



A PHARMACEUTICAL COMPOSITION FOR TREATING RHEUMATISM AND THE PREPARATION THEREOF

THE FIELD OF THE INVENTION

The invention is ~~about~~directed to a medicine ~~which is used to treat~~for treating rheumatism, and ~~its~~the medicine's preparation.

THE BACKGROUND OF THE INVENTION

It is believed that the rheumatoid and rheumatoid arthritis (RA) is refractory and about 18,000,000 RA patients have been disabled because of this disease. The medicine ~~research~~research for curing RA has continued about a century. Aspirin is the first medicine which is widely used to treat RA. The medicine to treat RA can be divided into 2 kinds: non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressive ~~agent~~agents. NSAIDs includes cyclophthasine, antinfan and adrenal cortex hormone. The clinical researchs have ~~proved~~proven the effectiveness of NSAIDs. The immuneosuppressive ~~agent~~agents ~~includes~~include methotrexate, cyclophosphane, penicillamine and et al. The ~~immunoregulation~~etc. Immunoregulation has become one of the important ~~therapies~~therapies in the recent years. But all the medicines which are used to treat rheumatism have serious side-effecteffects. The medicine which can treat rheumatism effectively and ~~non-poisonously~~with low toxicity hasn't been invented by ~~now~~before the present invention.

There are 3 directions in the research of ~~antirheumatic~~antirheumatics that should be emphasized. The first direction is NSAIDs and cytokine-antagon, such as recombined soluble

1 TNF α antagonist, IL-1 inhibitor and PAF inhibitor. The second direction is
 2 the new immunosuppressive agent and immunomodulator, such as
 3 cyclosporin A. The third direction is the compound medicines.

4 In the ~~TCM~~traditional Chinese medicine (TCM), the research on
 5 the “~~arthralgia disease~~”(equalsBi Zheng) (equal to the definition of
 6 rheumatism in the modern medicine) can be traced back to the Han
 7 dynasty more than 1,500 years ago. Three prescriptions: “Ma Xing Shi
 8 Gan decoction”, “Fangji ~~Huangqi~~Huangqi decoction” and “~~Wutong~~Wutou
 9 decoction”, which ~~is~~were used to treat “Bi Zheng” were recorded in the
 10 medicine classics “Shanghan Lun” ~~wrote~~written by the famous doctor
 11 Zhang Zhongjing at that time. ~~Gelsemium elegans Benth~~ is a A kind of
 12 wild plant in “huo ba hua”(Gelsemium elegans Benth) in the Sichuang
 13 province and it has been ~~proved~~proven effective to treat rheumatism in a
 14 clinical research carried ~~at~~out in the local area(Sichuang province). But
 15 the further study found that it had a serious side-effect on the
 16 ~~reproduction~~reproductive organs and some other uncontrollable
 17 ~~problem~~problems.

18 The treatment of “arthralgia disease” by the ~~method~~methods of
 19 TCM has reached a high level after development by numerous doctors’
 20 ~~development in so~~such a long-a history. By now, there are many effective
 21 prescriptions and herbs. There are more than 80 kinds of herbs and 29
 22 kinds of patent medicines recorded in the China pharmacopoeia 1995
 23 edition and 2000 edition. But there are still many problems, for example:
 24 ① the effect is not good enough in treating the serious arthralgia
 25 ~~disease~~diseases such as rheumatoid arthritis; ② the dosage forms ~~are~~can
 26 not ~~fit for~~meet the demands of modern life. ③ some medicine has good

1 ~~effect~~effects, but the side-effect ~~is~~effects are too serious ~~too~~, such as the
2 extract of *triperygium wilfordii*. So ~~that~~, it is necessary to develop
3 ~~the new~~ antirheumatic medicines that are highly-effective-lowly-noxious
4 ~~and, create minimal noxiousness, and are convenient for administration~~
5 ~~antirheumatic medicine~~. This medicine should have the similar
6 ~~effect~~effects and ~~the lower side-effect to the~~effects than synthetic
7 ~~artificial antirheumatic~~anti-rheumatic medicine.

8 9 THE CONTENT SUMMARY OF THE INVENTION

10 The invention ~~is to supply~~provides an antirheumatic, ~~which that~~ is
11 ~~highly-effective-lowly-noxious and, creates minimal noxiousness, is~~
12 convenient for administration, and its preparation thereof.

13 The ~~invented medicine's technical proposal is realized by~~
14 ~~using~~medicine uses the following crude herbs ~~as following~~:

15 *Tripterygium hypoglaucum* (Levl.) Hutch;

16 *Epimedium brevicornum* Maxim;

17 *Lycium barbarum* L; and,

18 *Cuscuta chinensis* Lam; (or *Cuscuta australis* R. Br.)

19 20 DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

21 The ~~invented medicine is made from the crude herbs~~
22 ~~above~~antirheumatic medicine of the present invention utilizes the crude
23 herbs as follows:

24 *Tripterygium hypoglaucum* (Levl.) Hutch;

25 *Epimedium brevicornum* Maxim;

26 *Lycium barbarum* L; and,

27 *Cuscuta chinensis* Lam (or *Cuscuta australis* R. Br.)

The ~~material~~crude herbs to produce the ~~invented~~antirheumatic medicine can be combined ~~on~~in several ways. The *tripterygium hypoglaucum* (Levl.) Hutch. is the necessary herb, one or two or three of the other three herbs can be added to ~~make~~make the ~~material~~compound prescription.

One of the optimal crude herbs rate of the ~~material~~compound prescription is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 1-4 ~~weightinweight~~parts by weight

Lycium barbarum L. 1-4 ~~weightinweight~~parts by weight

Cuscuta chinensis Lam. 1-4 ~~weightinweight~~parts by weight

The other optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 2 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 2 ~~weightinweight~~parts by weight

Lycium barbarum L. 1 ~~weightinweight~~parts by weight

Cuscuta chinensis Lam. 1 ~~weightinweight~~parts by weight

The third optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 ~~weightinweight~~parts by weight

Epimedium brevicornum Maxim. 1-4 ~~weightinweight~~parts by weight

The fourth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 2 ~~weightinweight~~parts by weight

weight

Epimedium brevicornum Maxim.

2 weightinweightparts by

weight

The fifth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch

1-4 weightinweightparts by

weight

Epimedium brevicornum Maxim

1-4 weightinweightparts by

weight

Lycium barbarum L

1-4 weightinweightparts by weight

The sixth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch

2 weightinweightparts by

weight

Epimedium brevicornum Maxim

2 weightinweightparts by

weight

Lycium barbarum L

1 weightinweightparts by weight

The seventh optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch

1-4 weightinweightparts by

weight

Epimedium brevicornum Maxim

1-4 weightinweightparts by

weight

Cuscuta chinensis Lam

1-4 weightinweightparts by weight

The eighth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch

2 weightinweightparts by

weight

Epimedium brevicornum Maxim

2 weightinweightparts by

weight

Cuscuta chinensis Lam 1 weight in weight parts by weight

The content of the ~~icariin~~icariin ($C_{33}H_{40}O_{15}$) in the medicine combinations above ~~can~~should not be less than 2.0 mg.

The optimal crude herbs rate of the material can be the other way as following:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 ~~weight in weight~~ parts by weight

Lycium barbarum L 1-4 weightinweightparts by weight

And / or *Cuscuta chinensis* Lam 1-4 ~~weightinweightparts by~~
weight

The optimal crude herbs rate of the material can be another way as following:

Tripterygium hypoglaucum (Levl.) Hutch 2 ~~weight in weight~~ weight parts by weight

Lycium barbarum L 1 weight in weight part by weight

And / or *Cuscuta chinensis* Lam 1 weight in weight part by weight

The crude herbs are prepared on the rate and then they can be made into any dosage forms used in the clinic, such as the bolus form, the powder forms, the ointment forms, the tablet forms, the ~~soft~~soft or hard capsule forms, the granule forms, the injection forms and so on.

The preparation method of the invented medicine is as following:

The crude herbs are prepared on the weight rate:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 weight in weight parts by
weight

Epimedium brevicornum Maxim 1-4 ~~weight in weight~~ parts by weight

Lycium barbarum L 1-4 weightinweightparts by weight

1 *Cuscuta chinensis* Lam 1-4 ~~weightinweight~~parts by weight

2 The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium*
 3 *brevicornum* Maxim are ~~smashed~~cut into pieces. Then the
 4 ~~powders~~pieces are decocted by water for 2 ~ 4 times separately. The
 5 *Lycium barbarum* L and *Cuscuta chinensis* Lam are soaked in the hot
 6 water (80~95°C) for 1 ~ 3 times separately. The ~~decocted~~decoction fluid
 7 and the immersion fluid of the herbs are collected and added to the
 8 ~~correspondent~~—~~macroscopic~~void~~corresponding~~ macroscopic void
 9 adsorbent ~~resins~~resin column separately. After the adsorption, the
 10 columns are washed with water until the flushing liquor ~~turns~~turns
 11 clear. Then the ~~columns~~resins are eluted by 60%-80% alcohol. The
 12 eluting liquors are collected from the time when its color ~~turning~~turns
 13 deep till the color ~~turning~~turns very weak. Then the alcohol in the ~~upper~~
 14 ~~part of the column~~ is pushed out by high pressure water and mixed with
 15 the eluting liquor. The total mixed eluting liquor is about 3 ~ 8 times
 16 ~~heavy~~concentrated of the correspondent crude herb. All the 4 eluting
 17 liquors are ~~reecycled~~recovered from the alcohol, and condensed to
 18 ~~the~~their specific density of 1.10 separately. The condensed liquors are
 19 ~~dehydrated~~dried by ~~spya~~spray drying method to get the extract of the
 20 crude herbs. The 4 ~~kind~~kinds of extracts are mixed uniformly to be made
 21 into any dosage ~~forms~~form that are needed by the clinic.

22 The optimal preparation method of the invented medicine is as
 23 ~~following~~follows:

24 The crude herbs are prepared on the weight rate:

25 *Tripterygium hypoglaucum* (Levl.) Hutch 2 ~~weightinweight~~parts by
 26 weight

Epimedium brevicornum Maxim 2 ~~weight~~in weight ~~parts by~~
weight

Lycium barbarum L 1 ~~weight~~in weight ~~part by weight~~

Cuscuta chinensis Lam 1 ~~weight~~in weight ~~part by weight~~

The *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Is smashed~~is cut into
pieces. Then the ~~powder~~pieces are added with 13, 10, 10 ~~fold~~times
 weight of the water to decoct ~~for~~3 times respectively. Each time is for 1
 hour. The *Epimedium brevicornum* Maxim is cut ~~to piece~~into pieces.
 Then the ~~herb~~pieces ~~is~~are added with 15, 10, 10 ~~fold~~times weight of
 the water to decoct ~~for~~3 times respectively. Each time is for 1 hour. The
Lycium barbarum L is smashed to ~~crude~~coarse powder and soaked in the
 hot water (80°C, 20 ~~fold~~times weight of the crude herb) ~~for~~3 times.
 Each time is for 1 hour. The *Cuscuta chinensis* Lam is smashed to
~~crude~~coarse powder and soaked in the hot water (80°C, 31 ~~fold~~times
 weight of the crude herb) ~~for~~3 times. Each time is for 1 hour. The
~~decocted~~decoction fluid and the immersion fluid of the herbs are filtrated
 separately and added to the correspondent ~~macroscopic~~void macroscopic
void adsorbent resins column JD-1 (WLD ~~resin column~~(the type of the
resin is JD-1 (WLD resin)) (manufactured by the Chinese Traditional
 Medicine Institute of Sichuan Province). Another useful resin is D₁₀₁,
 (manufactured by Nankai University Resin Factory, Tianjin). After the
 adsorption, the resins in the columns are eluted by 70% alcohol. The
 eluting liquors are collected from when its color ~~turning~~turns deep till
 the color ~~turning~~turns very weak. The alcohol is ~~recycled~~recovered from
 the eluting liquor. Then the rest of the liquor is condensed and
~~dehydrated~~dried to get the extract powder. The 4 ~~kind~~kinds of extract

1 powders are mixed uniformly to be made into any dosage forms that are
 2 needed by the clinic.

3 The invented medicine can be prepared ~~on~~by the method as
 4 ~~following~~follows:

5 The crude herbs are prepared on the weight rate which is recorded
 6 before. The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium*
 7 *brevicornum* Maxim are cut into pieces. The *Lycium barbarum* L and
 8 *Cuscuta chinensis* Lam are crushed or not. The 4 ~~kind~~kinds of herbs are
 9 extracted in ~~the~~ 0~95% alcohol **at 10 ~ 98°C** for 1~4 times separately or
 10 together. The extracted liquors are mixed or not. Then the extracted
 11 liquors are recovered from the alcohol, then condensed, ~~dehydrated~~dried,
 12 smashed and mixed uniformly or proportionally. The mixed powder can
 13 be made into any dosage form needed in the clinic.

14 The invented medicine can be made from the effective constituents
 15 of the 4 herbs.

16 The effective constituents of *Epimedium brevicornum* Maxim are
 17 ~~icariine~~icariin, icaraside I , icaraside II, and Icariin A. The effective
 18 constituents of *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Are~~are
 19 diterpenes, triterpenes and alkaloids ~~compound~~compounds. The effective
 20 constituents of *Lycium barbarum* L and *Cuscuta chinensis* Lam are both
 21 ~~flavone~~flavones.

22 ~~So that the~~The crude herb *Epimedium brevicornum* Maxim can be
 23 replaced by one or more kinds of the effective constituents of itself, such
 24 as ~~icariine~~icariin, icaraside I , icaraside II, and Icariin A. The crude herb
 25 *Tripterygium hypoglaucum* (Levl.) Hutch. ~~Can~~can be replaced by one or
 26 more kinds of the effective constituents of itself, such as diterpenes,

triterpenes and alkaloids ~~compound~~compounds. While the *Lycium barbarum* L and *Cuscuta chinensis* Lam can be replaced by ~~flavone~~flavones.

It has been ~~proved~~proven by the pharmacodynamics research that the invented medicine (Fengshiping Capsule) could inhibit the primary and secondary injury adjuvant arthritis (AA). It could inhibit the delayed hypersensitivity (DTH) in the ear of ~~the~~a mouse caused by the 2,4 dinitrofluorobenzene (DNFB). It could inhibit the antibody ~~produce~~production of the hemolysin and the activity of the IL-1, IL-2, IL-6 and TNF in the macrophage and splenocyte. The Fengshiping Capsule could inhibit the lymphocyte transformation induced by the ConA. It could inhibit the CD₄、CD₈ cells remarkably, especially CD₄ cells, but it can not affect the rate of CD₄/CD₈ ~~was not affected~~ very much. There was a remarkable linear relationship between the dosage and the effect. 12~18g (crude medicine)/kg was the minimum effective dose. The invented medicine could inhibit the activity of the NK cells. In the effective dose, the Fengshiping Capsule did not cause the atrophy of the important immune organs such as thymus and spleen, and did not inhibit the phagocytic activity of the macrophage.

The invented medicine had a remarkable ~~antiinflammatory~~anti-inflammatory action. It could inhibit the over penetrating condition of the capillary in the mouse's abdominal cavity caused by the ~~ethanoie~~injection of acetic acid ~~injected~~. It could ~~improve~~inhibit the swelling in the ear of the mouse caused by the croton oil. It could inhibit the pleuritis in the mouse and the assembling of the WBC to the CMC cyst in the rat induced by the carrageenan. But the invented medicine

1 couldn't obviously inhibit the rat's foot swelling induced by the
 2 carrageenan and the granuloma caused by the tampon-~~obviously~~. The
 3 Fengshipng Capsule could remarkably inhibit the body-twist reaction
 4 caused by the ~~ethanoieacetic~~ acid in the mouse-~~remarkably~~.

5 Experimental example 1: the effect on the adjuvant arthritis (AA)

6
 7 1.1 The preventing effect on the AA of the invented medicine

8 72 isogenous SD rats of the same batch, half male and half female,
 9 180 ~ 220g weight each, were divided randomly into 6 groups. Each
 10 group has 12 rats. ~~Every~~Each 6 rats lived in a cage. The perimeter of the
 11 double ~~ankle joints~~sankle joints and the feet of the ~~rat~~rats were measured
 12 accurately and recorded as the normal value. All the rats were ~~drenched~~
 13 ~~by~~given the same volume of the invented medicine ~~on the correspondent~~
 14 ~~concentration or in different concentrations of~~ the solution of the
 15 ~~Xihuangqi by the gastic injection~~ragacanth orally. 1 hour later, all the
 16 rats were injected with 0.1ml Freund's complete adjuvant (FCA) under
 17 the skin of the left postpedes. In the next 30 days, all the rats were
 18 ~~drenched with~~given orally the correspondent medicine once a day ~~on at~~
 19 the same dosage. And ~~in on~~ these days, the rats ~~were measured of the~~
 20 perimeters of the double ~~ankle joints~~sankle joints and the feet of the rats
 21 were measured once a day. In this experiment, the swelling degree
 22 (Δ cm)~~equaled to~~ equals the difference value of the perimeters measured
 23 after the FCA injection and before the FCA injection. (See the result in
 24 table 1.1 and 1.2) At the end of the experiment, the major organs of the
 25 rats were weighted. (See the table 1.3, 1.4)

Table 1.1 The effect of the Fengshipping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Swelling degree (Δcm) | | | | | | |
|------------------------|----------------|-----------------------|------------|-----------|------------|------------|-------------|-------------|
| | | 1d | 2d | 3d | 9d | 12d | 14d | 16d |
| Control | - | 0.69±0.17 | 0.69±0.12 | 0.92±0.18 | 0.84±0.41 | 1.10±0.30 | 1.65±0.68 | 2.10±0.55 |
| Fengshipping | 7.5 | 0.74±0.12 | 0.66±0.074 | 0.83±0.13 | 0.77±0.27 | 1.11±0.45 | 1.34±0.53 | 1.91±0.61 |
| Fengshipping | 15 | 0.80±0.24 | 0.62±0.13 | 0.76±0.18 | 0.49±0.17* | 0.73±0.34* | 1.00±0.48* | 1.38±0.67* |
| Fengshipping | 30 | 0.75±0.19 | 0.67±0.19 | 0.87±0.28 | 0.63±0.22 | 0.73±0.34* | 0.82±0.43** | 1.05±0.53** |
| Tripterygium Hutch. | 5 | 0.72±0.11 | 0.68±0.16 | 0.91±0.18 | 0.66±0.23 | 0.88±0.29 | 1.03±0.36* | 1.37±0.33* |
| prednisone | 0.01 | 0.64±0.14 | 0.64±0.16 | 0.50±0.26 | 0.46±0.25 | 0.72±0.46* | 0.87±0.46** | 1.28±0.69* |

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | | | |
|------------------------|----------------|--------------------------------|-------------|-------------|-------------|-------------|-------------|
| | | 18d | 20d | 22d | 24d | 26d | 28d |
| Control | - | 2.18±0.44 | 2.05±0.46 | 2.00±0.46 | 2.04±0.57 | 1.92±0.65 | 1.83±0.67 |
| Fengshipping | 7.5 | 1.74±0.73 | 1.81±0.55 | 1.81±0.52 | 1.77±0.55 | 1.65±0.55 | 1.55±0.49 |
| Fengshipping | 15 | 1.32±0.59** | 1.28±0.58** | 1.34±0.61* | 1.33±0.67* | 1.20±0.64* | 1.08±0.58** |
| Fengshipping | 30 | 0.95±0.50** | 0.87±0.51** | 0.95±0.54** | 0.89±0.59** | 0.90±0.57** | 0.86±0.51** |
| Tripterygium Hutch. | 5 | 1.47±0.43** | 1.50±0.43** | 1.49±0.43* | 1.42±0.53* | 1.40±0.56* | 1.32±0.57 |
| prednisone | 0.01 | 1.18±0.7**6 | 1.03±0.67** | 1.05±0.69* | 0.90±0.64** | 0.86±0.65** | 0.85±0.59** |

Comparing to the control group *P<0.05 , **P<0.01 (the signs have the same meaning in the following tables)

1.2 The effect of the Fengshipping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\bar{X} \pm S$) after the

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | | |
|---|----------------|--------------------------------|-----------|------------|-------------|-------------|
| | | 2d | 9d | 12d | 14d | 18d |
| Control | - | 0.14±0.05 | 0.06±0.10 | 0.34±0.36 | 0.80±0.52 | 1.43±0.67 |
| Fengshipping | 7.5 | 0.18±0.06 | 0.10±0.14 | 0.26±0.36 | 0.82±0.52 | 1.31±0.64 |
| Fengshipping | 15 | 0.15±0.08 | 0.02±0.06 | 0.13±0.10* | 0.37±0.31* | 0.90±0.56* |
| Fengshipping | 30 | 0.18±0.09 | 0.06±0.06 | 0.16±0.08* | 0.29±0.20** | 0.49±0.41** |
| Tripterygium hypoglaucom (Levl.) Hutch. | 5 | 0.16±0.07 | 0.01±0.07 | 0.11±0.10 | 0.44±0.19** | 0.87±0.56* |
| prednisone | 0.01 | 0.20±0.06 | 0.08±0.08 | 0.21±0.16 | 0.44±0.43 | 0.99±0.63 |

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | | |
|---|----------------|--------------------------------|-------------|-------------|-------------|-------------|
| | | 20d | 22d | 24d | 26d | 28d |
| Control | - | 1.28±0.57 | 1.38±0.64 | 1.35±0.75 | 1.20±0.78 | 1.12±0.63 |
| Fengshipping | 7.5 | 1.33±0.71 | 1.31±0.73 | 1.27±0.73 | 1.16±0.73 | 1.07±0.65 |
| Fengshipping | 15 | 1.74±0.57* | 1.92±0.61* | 0.95±0.64* | 0.88±0.58* | 1.83±0.55 |
| Fengshipping | 30 | 0.27±0.30** | 0.34±0.31** | 0.32±0.33** | 0.31±0.32** | 0.34±0.32** |
| Tripterygium hypoglaucom (Levl.) Hutch. | 5 | 0.82±0.65* | 0.89±0.70* | 0.80±0.67* | 0.83±0.68 | 0.75±0.69 |
| prednisone | 0.01 | 0.82±0.72* | 0.79±0.74* | 0.75±0.67** | 0.68±0.64* | 0.71±0.67 |

1.3 The effect of the Fengshipng on the body weight of the AA rats ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Body weight change(g) | | |
|--|-------------|-----------------------|---------------------|-----------|
| | | Initiative BW | BW at 1 month later | BW change |
| Control | - | 228±34 | 231±52 | 3 |
| Fengshiping | 7.5 | 229±34 | 220±46 | -9 |
| Fengshiping | 15 | 223±40 | 232±34 | 9 |
| Fengshiping | 30 | 224±37 | 256±60 | 32 |
| Tripterygium hypoglaucum (Levl.) Hutch. prednisone | 5 0.01 | 226±45 264±55 | 230±43 244±31 | 4 -21 |

1.4 The effect of the Fengshiping on the organ weight of the immune system in the AA rats (prevention experiment)($\bar{X} \pm S$)

| Group | Dose (g/kg) | Organ index [(organ weight/body weight)/100] | | | |
|--|-------------|---|------------------------|--------------------------------|-----------------------------|
| | | Liver | Spleen | Thymus | adrenal gland |
| Control | - | 3.92±0.65 | 0.34±0.10 | 0.098±0.040 | 0.027±0.01 |
| Fengshiping | 7.5 | 3.73±0.29 | 0.31±0.09 | 0.078±0.038 | 0.027±0.008 |
| Fengshiping | 15 | 3.48±0.32 | 0.38±0.10 | 0.100±0.034 | 0.023±0.005 |
| Fengshiping | 30 | 3.38±0.28* | 0.44±0.12* | 0.100±0.032 | 0.022±0.007 |
| Tripterygium hypoglaucum (Levl.) Hutch. prednisone | 5 0.01 | 3.21±0.30** 3.04±0.20** | 0.36±0.05 0.32±0.08 | 0.052±0.011** 0.050±0.060** | 0.026±0.009 0.020±0.004* |

1.2 The therapeutic effect on the AA of the invented medicine

50 male SD rats were divided into 5 groups at random. The model building was the same as the prevention experiment, but the correspondent medicines were drenched given orally 13 days after the injection of the FCA. The medicines were drenched given once a day for 2 weeks. The swelling degree (Δ cm) was the difference of the perimeters between the value of first administration day and the other days. (See the result in table 1.5, 1.6) The major organs' weight is showed in table 1.7.

**1.5 The therapeutic effect of Fengshiping on the swelling degree of
The left anklejoint and foot in the AA rats ($\bar{X} \pm S$)**

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | |
|-------------|----------------|--------------------------------|-------------|-------------|-------------|
| | | 1d | 2d | 4d | 6d |
| Control | - | 1.81±0.27 | 1.92±0.19 | 2.12±0.22 | 2.16±0.27 |
| Fengshiping | 7.5 | 1.68±0.50 | 1.64±0.54 | 1.70±0.57 | 1.82±0.61 |
| Fengshiping | 15 | 1.44±0.41* | 1.51±0.36** | 1.65±0.34** | 1.74±0.31** |
| Fengshiping | 30 | 1.50±0.56 | 1.48±0.41** | 1.51±0.44** | 1.59±0.51** |
| prednisone | 0.01 | 1.78±0.51 | 1.70±0.51 | 1.63±0.50* | 1.58±0.50** |

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | |
|-------------|----------------|--------------------------------|-------------|-------------|-------------|
| | | 8d | 10d | 12d | 14d |
| Control | - | 1.92±0.32 | 1.87±0.34 | 1.92±0.39 | 1.78±0.44 |
| Fengshiping | 7.5 | 1.67±0.68 | 1.60±0.71 | 1.61±0.77 | 1.58±0.71 |
| Fengshiping | 15 | 1.46±0.37** | 1.48±0.30* | 1.28±0.37** | 1.22±0.38** |
| Fengshiping | 30 | 1.29±0.58** | 1.29±0.65** | 1.26±0.67** | 1.20±0.68* |
| prednisone | 0.01 | 1.27±0.46** | 1.09±0.54** | 0.94±0.50** | 0.94±0.42** |

**1.6 The therapeutic effect of Fengshiping on the swelling degree of
the right anklejoint and foot in the AA rats ($\bar{X} \pm S$)**

| Group | Dose (g/kg) | Swelling degree (Δ cm) | | | |
|-------------|----------------|--------------------------------|-------------|-------------|--------------|
| | | 2d | 4d | 6d | 8d |
| Control | - | 0.36±0.26 | 0.45±0.25 | 0.55±0.34 | 0.47±0.29 |
| Fengshiping | 7.5 | 0.12±0.25 | 0.34±0.32 | 0.48±0.41 | 0.28±0.38 |
| Fengshiping | 15 | 0.21±0.18 | 0.38±0.27 | 0.44±0.33 | 0.21±0.33* |
| Fengshiping | 30 | 0.10±0.48 | 0.06±0.28** | 0.11±0.24** | 0.06±0.27** |
| prednisone | 0.01 | 0.10±0.13* | 0.15±0.28* | 0.11±0.25** | -0.08±0.34** |

| Group | Dose (g/kg) | Swelling degree(Δ cm) | | |
|-------------|----------------|-------------------------------|--------------|--------------|
| | | 10d | 12d | 14d |
| Control | - | 0.48±0.25 | 0.46±0.31 | 0.40±0.36 |
| Fengshiping | 7.5 | 0.35±0.30 | 0.30±0.29 | 0.30±0.35 |
| Fengshiping | 15 | 0.19±0.45* | 0.06±0.31** | -0.06±0.34** |
| Fengshiping | 30 | 0.02±0.39** | 0.05±0.38* | -0.02±0.41** |
| prednisone | 0.01 | -0.13±0.28** | -0.26±0.36** | -0.33±0.39** |

n = 10 , comparing with the control group , *P<0.05 , **P<0.01

1.7 The effect of the Fengshiping on the organ weight of the immune system in the AA rats ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Organ index [(organ weight/body weight)/100] | | | |
|---|----------------|--|-------------|--------------|---------------|
| | | Liver | Spleen | Thymus | adrenal gland |
| Control | - | 0.35±0.23 | 0.35±0.061 | 0.073±0.014 | 0.026±0.0071 |
| Fengshiping | 7.5 | 3.21±0.52 | 0.33±0.091 | 0.071±0.026 | 0.024±0.0085 |
| Fengshiping | 15 | 3.40±0.54 | 0.36±0.014 | 0.067±0.022 | 0.023±0.0048 |
| Fengshiping | 30 | 2.79±0.43 | 0.32±0.083 | 0.069±0.029 | 0.023±0.0072 |
| Tripterygium hypoglaucomum (Levl.) Hutch. | 5 | 3.92±0.59 | 0.35±0.100 | 0.075±0.034 | 0.027±0.0060 |
| prednisone | 0.01 | 3.52±0.35 | 0.28±0.047* | 0.05±0.011** | 0.02±0.0043* |

The data ~~showed~~shown in the ~~table~~tables 1.1, 1.2, 1.3, 1.5 and 1.6 ~~proved~~proves that the Fengshiping could strongly inhibit the primary and secondary injury caused by FCA, whenever the medicine was ~~drenched~~given at the beginning of the FCA injection or 2 weeks after the FCA injection. The experiments ~~proved~~prove that the Fengshiping ~~had~~has both the preventing and the therapeutic effect. By comparing the effect of Fengshiping on the swelling degree in the anklejoint and foot, we found that the Fengshiping could inhibit the specific ~~immunoswelling~~immuno-swelling in the ~~anklejoint~~ankle joint better than the nonspecific ~~immunoswelling~~immuno-swelling in the foot of rats. This result ~~indicated~~indicates that the main effect of Fengshiping was inhibiting the immunity inflammatory reaction.

The data in the ~~table~~tables 1.3, 1.4 and 1.7 ~~showed~~show that the AA rats had no obvious BW increase during the period of the experiment. In the group ~~drenched of~~given the Fengshiping ~~on~~with the effective dosage, the rats still had BW increase. In the groups of prednisone and

preventing, the BW of rats had decreased, while the thymus and adrenal gland were atrophied. In the group of *tripterygium hypoglaucum* (Levl.) Hutch, the thymus had ~~thinned~~not atrophied yet. But in the 3 groups ~~drenched with~~given the different dosage of Fengshiping, ~~no~~no atrophy of the thymus and adrenal gland were observed.

1.3 The pathologic change of the AA after the treatment of the invented medicine in rats

45 SD rats, 180 ± 20 g weight each, were divided into 6 groups. After the AA caused by FCA appeared, all the rats were ~~drenched with~~given orally Fengshiping solution ~~by gastric injection~~ for 5 days once a day. 1 hour after the last administration, the joint index of the rats was ~~measured~~evaluated and calculated. The secondary injured postpedes' joints on the opposite of the FCA injection were taken off and soaked in the formaldehyde. After the tissues were HE tinted, the pathological change of the synovium and cartilage were observed and recorded. The data ~~were showed~~are shown in table 1.8.

1.8 The effect of Fengshiping on the AA joint index in the rats ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Rat number | Joint index |
|----------------------------------|-------------|------------|----------------------|
| Control | - | 8 | 0** |
| AA model | - | 7 | 6.2 ± 0.49 |
| Fengshiping | 7.5 | 9 | $4.86 \pm 0.90^{**}$ |
| Fengshiping | 15 | 7 | $4.71 \pm 0.95^{**}$ |
| Fengshiping | 30 | 7 | $4.56 \pm 1.13^{**}$ |
| Glucosidorum Tripterygll Totorum | 0.006 | 7 | $4.57 \pm 0.79^{**}$ |

Comparing with the model group** $P < 0.01$

The joint index was the sum of the inflammatory scores of the four

limbs. According to the degree of inflammatory, each limb was evaluated on the criteria as following: normal (0), red without swelling (1), red and swelling (2), seriously swelling (3), deforming and ~~tetanus~~stiffness (4).

Observed from the microscope, the joint synovial membranes of the rat posterior limb were hyperplasia in the model group; ~~and~~ the collagen fiber had increased; and there was infiltration of lymphocytes and plasma cells in the tissue. ~~The~~An obvious granuloma had formed. The synovial cells had degenerated and the cytochylema had been tinted red; the caryon had been pycnosis; the epithelium had exfoliated in some part of the synovial membrane. The cartilage ~~turned atrophy~~became atrophied; the surface of it was rough and some of the chondrocytes had proliferated lightly.

After the treatment ~~of~~with the Fengshiping, the inflammation of the joint synovial membrane was inhibited, more collagen fiber was produced; less synovial cells exfoliated ; the cells on the surface of the cartilage had proliferated and the surface had turned smooth. The cartilage was ~~on the~~in a recovering condition.

Experimental example 2: The effect of Fengshiping on the delayed hypersensitivity reaction (DTH) caused by 2,4-DNFB in the ear of the mouse

50 NIH mice, half male and half female, were divided into 5 groups. Each mouse was led ~~to~~into a hypersensitivity reaction by using the 1% DNFB acetone solution ~~on the~~at a dosage of 0.025ml at the right place of the abdomen where the ~~pilus~~fur had been ~~cut yet~~removed. Using the same solution on the same place ~~was to enhance~~enhanced the

hypersensitivity reaction on the third day. On the fifth day, all the mice were smeared with the 1% DNFB edible oil solution at the mice's right ears ~~on the~~ at a dosage of 0.01 ml each. 24 hours later, all the mice were killed. The mouse's 2 ears were ~~weighted~~ weighed by the torsion balance and the difference of the 2 ears was recorded as the DTH degree caused by the DNFB. The experiment was carried out on the different immune and administration processes.

2.1 The effect on the DTH by the full course administration

The immune and administration processes is as ~~following~~ follows:

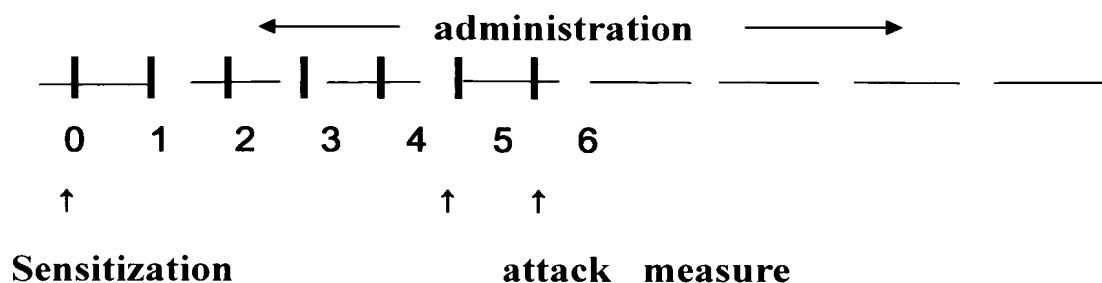


Table 2.1 The effect of Fengshipping on the DTH caused by DNFB in the NIH mouse ($\bar{X} \pm S$)

| group | dose (g/kg) | Administration time (day) | Mice number | Percent of ear swelling | Percent of inhibition (%) | P value |
|------------------------------|----------------|------------------------------|-------------|-------------------------|------------------------------|---------|
| control | | | 10 | 34.20±3.77 | | |
| Fengshipping | 27 | 0~5 | 10 | 26.24±3.34 | 23.3 | <0.01 |
| Fengshipping | 40 | 0~5 | 10 | 12.99±4.96 | 62.0 | <0.01 |
| Fengshipping | 60 | 0~5 | 10 | 10.43±7.53 | 69.5 | <0.01 |
| cortisumman | 0.003 | 0~5 | 10 | 13.93±4.41 | 59.3 | <0.01 |
| control | | | 10 | 42.43±5.28 | | |
| Fengshipping | 40 | -2~0 | 10 | 31.50±10.52 | 25.0 | <0.01 |
| Fengshipping | 40 | -2~2 | 10 | 30.88±7.92 | 27.2 | <0.01 |
| Fengshipping | 40 | -2~5 | 10 | 21.07±4.62* | 50.3 | <0.01 |
| Fengshipping | 40 | 5~6 | 10 | 32.00±9.37 | 41.7 | <0.01 |
| cyclophosphane | 0.05 | -2~2 | 10 | 39.40±10.78 | 8.1 | >0.05 |
| cyclophosphane | 0.05 | -2~0 | 10 | 37.47±6.71 | 11.7 | >0.05 |
| control | | | 10 | 38.50±4.67 | | |
| cyclophosphane | 0.1×3 | 0、2、4 day once a day | 10 | 23.00±7.65 | 40.3 | <0.01 |
| cyclophosphane | 0.25 | -3d | 10 | 41.84±7.75 | -8.7 | |
| Fengshipping | 60 | 0~4 | 10 | 27.20±10.20 | 29.4 | <0.01 |
| cyclophosphane +Fengshipping | 0.25 + 60 | -3,0~4 | 10 | 38.07±6.65 | 1.1 | |

*comparing with the other groups $P<0.05$ 或 $P<0.01$

1 According to the data ~~showed~~shown in table 2.1, it indicated that
2 the Fengshiping had ~~an~~an obvious inhibiting effect on the DTH caused by
3 DNFB. There was a significant relationship between the dosage and the
4 effect. The inhibiting activity ~~increases~~increased when the dosage
5 ~~adds~~increased. The inhibiting percent could reach 69.5% on the dosage
6 of 60.9g/kg.

7 8 **2.2** The effect on the DTH of the different administration time

9 The immune and administration processes and the
10 ~~correspondent~~corresponding results ~~had~~have been ~~showed~~shown in the
11 middle and bottom parts of ~~the~~ table 2.1. According to the results
12 showed in the middle part of the table 2.1, all the administration ways
13 could significantly inhibit the DTH of the mouse in spite of the
14 administration beginning from the 2 days before the sensitization ~~and to~~
15 ending at the sensitization, or beginning from the 2 days before the
16 sensitization ~~and to~~ ending 2 days after the sensitization, or beginning
17 from the 2 days before the sensitization ~~and to~~ ending 5 days after the
18 sensitization, or beginning before the attack and ending after the attack.
19 But the administration way that began 2 days before the sensitization
20 and ended 5 days after the sensitization had the most powerful inhibiting
21 activity. It indicated that the Fengshiping could inhibit the DTH by a
22 compound mechanism that it could inhibit the cells
23 ~~participant~~participating in the early period of the DTH, the effector cells
24 in the advanced period and the cells related to the DTH in the middle
25 period. This mechanism was different from that of the cyclophosphane.
26 On a small dosage, the cyclophosphane didn't affect the DTH, if its
27 administration way was ~~beginning~~method began from the 2 days before

the sensitization and ~~ending~~ended at the sensitization day or 2 days after the sensitization day.

Based on the bottom part of the table 2.1, if a high dosage of cyclophosphane was ~~drenched~~given to the mouse ~~in~~at one time 3 days before the sensitization, the function of the Th cells would turn sthenic because of the powerful inhibition on the Ts cells. The DTH in the mouse would be enhanced. If the cyclophosphane was used with the Fengshiping ~~on~~in this administration ~~way~~method, it could lower the inhibiting activity of Fengshiping. This result indicated that the Fengshiping ~~have~~has a different ~~maehnizm~~machnism to the cyclophosphane in the control of DTH. The Fengshiping ~~maybe~~hadmay have a higher activity in inhibiting the THcells.

Experimental example 3: The effect on the humoral immunity

3.1 The effect on the ~~produce~~product of the hemolysin antibody caused by the chick RBC

190 mice, 18-22g weight, half male and half female, were divided into 19 groups. Each mouse was immunized with 5% CRBC solution 0.2 ml. The Fengshiping solutions were ~~drenched~~given orally to the mice at the different times. 7 days after the immunization, all the mice were sampled using the blood from the eyes. Then the blood samples were diluted and ~~measured~~ the level of the hemolysin antibody was measured. The results ~~were showed~~are shown in table 3.1, 3.2 and 3.3.

Table 3.1 The effect of Fengshiping on the produce of the hemolysin antibody in the NIH mouse ($\bar{X} \pm S$)

| group | dose | Administration | Mouse | Hemolysin | Inhibiting | P |
|-------|------|----------------|-------|-----------|------------|---|
|-------|------|----------------|-------|-----------|------------|---|

| | (g/kg) | time | number | value | percent (%) | value |
|-------------------------------------|--------|------|--------|------------------|-------------|--------|
| control | | | 10 | 169.0±62.0 | | |
| Fengshipping | 18 | 0~7 | 10 | 46.0±15.6 | 72.8 | <0.01 |
| Fengshipping | 27 | 0~7 | 10 | 35.4±12.0 | 79.1 | <0.01 |
| Fengshipping | 40 | 0~7 | 10 | 28.2±5.9 | 83.3 | <0.01 |
| Fengshipping | 60 | 0~7 | 10 | 16.7±3.0 | 90.1 | <0.01 |
| Tripterygium hypoglaucom (Levl.) | 13.3 | 0~7 | 10 | 121.0±88.0 ** | 28.4 | <0.015 |
| Hutch. cyclophosphane | 0.02 | 0~7 | 10 | 35.0±12.0 | 79.3 | <0.01 |

**** comparing with the Fengshipping (40g/kg) group P<0.01**

**Table 3.2 The effect of Fengshipping on the produce of the hemolysin
antibody in the ICR mouse ($\bar{X} \pm S$)**

| group | dose (g/kg) | Administration time | Mouse number | Hemolysin value | Inhibiting percent (%) | P value |
|-------------------------------------|----------------|------------------------|-----------------|-----------------|---------------------------|------------|
| control | - | - | 10 | 124.70±42.60 | | |
| Fengshipping | 12 | 0~7 | 10 | 75.00±53.10 | 39.9 | <0.05 |
| Fengshipping | 18 | 0~7 | 10 | 45.60±22.70 | 63.4 | <0.01 |
| Fengshipping | 27 | 0~7 | 10 | 29.10±22.10 | 76.8 | <0.01 |
| Fengshipping | 40 | 0~7 | 10 | 28.20±5.30 | 77.4 | <0.01 |
| Tripterygium hypoglaucom (Levl.) | 6.0 | 0~7 | 10 | 143.50±67.90** | | >0.05 |
| Hutch. cyclophosphane | 0.02 | 0~7 | 10 | 27.80±6.60 | 77.9 | <0.01 |

****comparing with the Fengshipping (18g/kg) group P<0.01**

**Table 3.3 The effect of Fengshipping on the produce of the hemolysin
antibody in the ICR mouse ($\bar{X} \pm S$)**

| group | dose (g/kg) | Administration time | Mouse number | Hemolysin value | Inhibiting percent (%) | P value |
|----------------|----------------|------------------------|-----------------|--------------------|---------------------------|------------|
| control | - | - | 10 | 256.0±26.0 | | |
| Fengshipping | 18 | -7~7 | 10 | 198.0±50.0 | 22.7 | <0.01 |
| Fengshipping | 18 | -3~7 | 10 | 156.0±85.0 | 39.1 | <0.01 |
| Fengshipping | 18 | 0~7 | 10 | 98.0±35.0 | 61.7 | <0.01 |
| cyclophosphane | 0.02 | 0~7 | 10 | 25.0±4.0 | 90.2 | <0.01 |

According to the data in table 3, it indicated that the Fengshipping

1 ~~had~~has a remarkable inhibiting effect on the ~~produce~~product of the
2 hemolysin antibody in the different mouse species and this effect would
3 increase along with ~~the~~an increase of the dosage. There was a certain
4 relationship between the dosage and the effect. The lowest effective
5 dosage was 12g/kg. ~~Comparing~~Compared with the same quantity of
6 Tripterygium hypoglaucum (Levl.) Hutch, the Fengshiping had a higher
7 inhibiting activity. Based on the data in table 3.1, the inhibiting activity
8 of Fengshiping was 2.25 times higher than the Tripterygium
9 hypoglaucum (Levl.) Hutch. The inhibiting activity of Tripterygium
10 hypoglaucum (Levl.) Hutch. ~~On~~with the dosage of 13.5g/kg was weaker
11 than that of the Fengshiping which ~~containing~~contains 6g/kg
12 Tripterygium hypoglaucum (Levl.) Hutch).

13 **3.2** The effect of the Fengshiping on the humoral immunity in the AA 14 mouse

15 The NIH mice, 20±2g weight, were injected with 0.05 ml FCA
16 under the vola skin of the right postpedes. 3 weeks ~~late~~later the AA
17 model mouse builded. The model mice were divided into 6 groups
18 randomly and ~~drenched with the correspondent~~given orally the
19 corresponding medicines for 5 days. At the beginning of the
20 administration, all the mice were sensitized with 0.5ml 10% sheep RBC
21 (SRBC). Five days later, all the mice were killed. Their spleens were
22 taken out and washed by the Hank's liquor to prepare the lymphocyte
23 suspended liquor. The concentration of the cells was adjusted to
24 2×10^7 / ml. 1 ml lymphocyte suspension, 1 ml 0.2% SRBC and 1 ml
25 1:30 addiment were added to one test tube. The tube was put in the water
26 bath at 37°C for 1 hour. Then the tube was centrifugated at 2000rpm for

5 minutes. The supernatant fluid was separated and tested its optical density at the 415nm wavelength on the 722 type spectrophotometer. The value was the ~~represent~~representative of PFC quantity.

The other share of the blood samples ~~got~~ from the sensitized mice was separated the serum to test the potency of the antibody. The measured data were recorded on the way of Log2 value. (See the data in table 3.4)

Table 3.4 The effect of Fengshiping on the humoral immunity in the mouse ($\bar{X} \pm S$)

| group | dose (g/kg) | Mouse number | PFC (OD) | IgM(Log2) |
|----------------------------------|--------------------|-----------------|----------------|---------------|
| control | - | 8 | 0.819±0.013# | 6.875±0.641 |
| AA model group | - | 10 | 0.940±0.019** | 7.700±0.599* |
| fengshiping | 5 | 8 | 0.834±0.012**# | 6.875±0.641# |
| fengshiping | 10 | 8 | 0.834±0.012**# | 6.750±0.886# |
| fengshiping | 20 | 8 | 0.830±0.014**# | 6.375±0.518## |
| Glucosidorum Tripterygll Totorum | 0.012 | 10 | 0.835±0.015**# | 6.950±0.597# |

Comparing with the control group *P<0.05, **P<0.01; comparing with the model group # P<0.05, ## P<0.01

According to the table 3.4, the levels of PFC and IgM in the AA mouse were higher than that of the normal mouse. The Fengshiping could lower the ~~produce~~value of the PFC and IgM in the AA mouse significantly.

Experimental example 4: The effect of the Fengshiping on the passive cutis anaphylactic reaction (PCA) in the rat.

The rats were injected with the egg albumn at 10mg/kg in the muscle. At the same time, all the rats were injected with 2×10^{10} (0.2ml) bordetella pertussis in the abdominal cavity for sensitization. 2 weeks later, all the rats were killed to sample the blood. All the blood samples were separated for preparing the serum.

60 rats, 150~200g, half male and half female, were divided into 6 groups at random. In the light narcosis condition induced by ~~aether~~ether, each rat was ~~cut the fleece in the~~shaved on its back and injected with the 2 concentrations of anti-egg-album serum 0.1ml under the skin at the ~~fairless~~shaved place. The serums were diluted ~~onto~~ the ~~rates~~concentrations of 1:5(d1) and 1:10(d2) before the experiment. 48 hours later, all the rats were ~~attacked by~~ intravenous injecting intravenously injected with the 0.5% ~~evens~~Evans blue normal saline solution 1 ml which ~~containing~~contains 1 mg egg albumin. 20 minutes later, the rats were killed by decapitation. The rats' back ~~skins~~skins were dissected and turned over. According to the ~~dark and light~~dark and ~~area~~light and areas of the blue stains exudated from the vessels, all the rats were evaluated by several people. The ~~skins~~skins stained by the ~~evens~~Evans blue were scissored and soaked in 5ml 0.1% sodium sulfate acetone (7:3) solution for 48 hours. Then it was ~~centrifugated~~centrifuged to separate the supernatant liquor. The optical density of the supernates ~~were~~was ~~measured the optical density~~ at the wavelength 590nm to calculate the degree of the PCA reaction and the inhibiting percent. The results were shown in table 4.

Table 4 The effect of Fengshiping on the PCA in rat ($\bar{X} \pm S$)

| Group | dose (g/kg) | value | | absorbancy | |
|-------|--------------------|----------------|----------------|----------------|----------------|
| | | d ₁ | d ₂ | d ₁ | d ₂ |

| | | | | | |
|---|-----|-------------|-----------|---------------|--------------|
| Control | - | 5.60±1.78 | 2.40±2.46 | 0.191±0.129 | 0.096±0.106 |
| Fengshiping | 12 | 7.50±2.51 | 4.20±2.49 | 0.402±0.213* | 0.192±0.175 |
| Fengshiping | 24 | 7.10±2.13 | 4.10±1.79 | 0.310±0.177 | 0.137±0.099 |
| Fengshiping | 48 | 6.00±1.83 | 1.70±1.95 | 0.121±0.109 | 0.024±0.026* |
| Tripterygium hypoglaucum (Levl.) Hutch. | 8 | 6.11±1.27 | 2.56±1.67 | 0.223±0.122 | 0.074±0.045 |
| Ketotifen | 0.1 | 2.78±1.64** | 0.67±1.41 | 0.033±0.024** | 0.027±0.019* |

Comparing with the control group *P<0.05 , **P<0.01

According to the table 4, it is indicated that the Fengshiping had a weak effect on the PCA in the rat. Only ~~on~~at a high dosage, the inhibiting effect of Fengshiping was obviously different from that of the control group.

Experimental example 5: The effect of Fengshiping on the cytokines.

5.1 The effect of Fengshiping on the levels of TNF α and IL-2 in the mouse.

60 ICR mice, 18~22g, half male and half female, were divided into 6 groups at random. Each group was ~~drenched~~of given orally the ~~correspondent~~corresponding medicines including the different dosages of Fengshiping and the other medicines. The medicines were administrated once a day for 10 days. 24 hours after the last administration, samples from the mice were ~~sampled~~taken, including the macrophage and spleen cells from the abdominal cavity in the aseptic condition. The samples were washed with Hank's liquor ~~for 2 times~~twice and non-serum RPIM 1640 liquor ~~for 1 time~~once. Then the washed samples were diluted to the suspension with the 5% FCS-RPMI 1640 at the concentration of 2×10^8 / ml. Then the suspensions were added with 10ng/ml LPS or the 10ng/ml ConA and cultured in the 5% CO₂

1 condition for 48 hours at 37°C. Then the cultured ~~suspension were~~
2 ~~measured the suspensions'~~ TNF α and IL-2 levels ~~on~~ were measured using
3 the usual methods.

4 The measurement of TNF α

5 The ~~batten~~ plate was coated by mouse TNF- α monoclonal antibody.
6 The ~~batten was added with the~~ plate had cultured supernate ~~on~~ added at
7 the dose of 50 μ l/ hole. Then the ~~batten~~ plate was ~~put~~ kept still for 60
8 minutes at the room temperature. Then the ~~batten~~ plate was ~~added~~ mixed
9 with biotin antibody ~~mark~~ marker at 25°C for 2 hours. Then the enzyme
10 labeled avidin was added into the ~~batten~~ plate and left for 30 minutes.
11 After adding the substrate constant for 30 minutes, the ~~batten was added~~
12 ~~with the stop liquor~~ was added to the plate. The mixed liquor was
13 measured using the OD value at the wavelength of ~~the~~ 450nm. The
14 content of the TNF- α (ng/ml) was calculated on the data of OD value by
15 the method of standard curve.

16 The measurement of the IL-2:

17 The CTLL cells which ~~was~~ were on the logarithmic growth phase
18 and whose growth depends on the IL-2, were adjusted to the suspension
19 at the concentration of 1×10^5 /ml with the 5% FCS-RPMI 1640. Then the
20 96 hole cell culturing ~~batten were added~~ plate was filled with the CTLL
21 cell suspension ~~on~~ to the quantity of 100 μ l/hole. The supernates were
22 added ~~on~~ to the quantity of 100 μ l/hole and each sample was added to 3
23 holes. The samples cultured were compared with the different dilutions
24 of standard rHIL-2 and the control sample (culture fluid) to measure the
25 IL-2. All the samples were cultured in the 5% CO₂ for 24 hours at 37°C.
26 6 hours before the end of the culture, all the samples were centrifuged

and separated from the supernate. Each hole ~~were taken out~~had 110 μ l of supernate removed and ~~added with~~then 10 μ l of MTT was added. The samples were cultured for 3 hours at 37°C, and then the OD was measured ~~the OD~~ at the ~~wavelength~~wavelengths of 570nm and 630nm. The final OD value of the sample was the difference of OD (570nm) and OD (630nm).

$$\text{IL-2 activity} = \frac{\text{Sample } \overline{\text{OD}} - \text{Control (Culture Fluid)} \overline{\text{OD}}}{\text{Standard Sample } \overline{\text{OD}} - \text{Control (Culture Fluid)} \overline{\text{OD}}} \times \text{activity of the standard sample (IU/ml)}$$

Table 5.1 The effect of Fengshiping on the TNF α and IL-2 ($\bar{X} \pm S$)

| group | dose (g/kg) | Mouse number | TNF (pg/ml) | IL-2 (IU/ml) |
|---|-------------|--------------|---------------------|--------------------|
| Control | - | 10 | 87.80 \pm 14.63 | 26.30 \pm 4.22 |
| | 12 | 10 | 62.14 \pm 13.13** | 16.00 \pm 2.89** |
| Fengshiping | 24 | 10 | 58.60 \pm 9.63** | 18.80 \pm 2.86** |
| | 36 | 10 | 54.40 \pm 10.88** | 18.20 \pm 2.86** |
| Tripterygium hypoglaucom (Levl.) Hutch. | 8 | 10 | 58.25 \pm 10.32** | 16.00 \pm 2.88** |
| cyclophosphane | 0.02 | 10 | 42.20 \pm 9.57** | 10.10 \pm 3.00** |

*P<0.05 , **P<0.01

According to the data in Table 5.1, it suggested that the Fengshiping have a obvious inhibiting effect on the TNF α . On the dosage of 12g/kg, the medicine had showed aan obvious inhibiting effect. Along with the increase of the dosage, the inhibiting effect increased. But the dosage-effect curve went gentlyevenly. The Fengshiping had an obvious inhibiting effect on the IL-2, ~~atbut~~ no dosage-effect relationship was

1 observed.

2 **5.2** The effect of Fengshiping on the IL-1, IL-6

3 70 NIH mice, 18-22g weight, half male and half female, were
4 divided into 7 groups at random. All the groups were ~~drenched~~
5 ~~with~~given orally the ~~correspondent~~corresponding medicines
6 (fengshiping and the other medicines). The medicines were
7 ~~drenched~~given once a day for 10 days. 24 hours after the last
8 administration, all the mice were killed and ~~sampled~~ the macrophage and
9 spleen cells from the abdominal cavity were sampled. The IL-1 and IL-
10 6 in the samples were measured.

11 The measurement of IL-1:

12 The macrophages in the abdominal ~~ear~~cavity were sampled in
13 the asepsis condition. Then the samples were washed by the Hank's
14 liquor ~~for~~ 2 times and nonserum RPMI1640 liquor ~~for~~ 1 time. Then the
15 clear samples were adjusted to the 4×10^6 / ml cell suspension with 5%
16 FCS-RPMI liquor. 1 ml of the suspension was added to the test tube and
17 cultured at 37°C for 1 hour. The unadherent cells were abandoned. Then
18 the cultured liquor was added with 5% FCS-RPMI 1640 and LPS
19 (10ng/ml) to culture. The cells were cultured in 5% CO₂ at 37°C for 72
20 hours. During the course, the cultured cells were freezed and thawed ~~for~~
21 several times. The final product was saved at 4°C. The C57 ~~mice were~~
22 ~~sampled the thymus~~mices' thymuses in the asepsis condition were
23 sampled. Then the samples were prepared to the 1×10^6 /ml cell
24 suspension with 5% FCS-RPMI1640.

25 100μl supernate separated from the frost thawing liquor and 100μl
26 cell suspension of thymus were added into the 96-hole flat bottom cell-

1 culture ~~battenplate~~. Each sample was cultured in 3 holes and compared
 2 with the different dilutions standard rHIL-1 and the control sample
 3 (culture fluid). Each hole ~~was added with~~had 2ng ConA added and then
 4 the ~~battenplate~~ was cultured in the 5% CO₂ at 37°C for 72 hours. 14
 5 hours before the end of the culture, each hole ~~was added with~~had 3H-
 6 TdR 0.1μCi added. The cultured cells were collected with a multihead
 7 cell-harvesting apparatus and ~~measured~~ the cpm value was measured.

$$\begin{aligned} \text{IL-1 activity} = & \frac{\text{Sample cpm} - \text{Control (Culture Fluid) cpm}}{\text{Standard Sample cpm} - \text{Control (Culture Fluid) cpm}} \\ & \times \text{activity of the standard (ng/ml)} \end{aligned}$$

13 The measurement of the IL-6:

14 The spleen cells were sampled in the asepsis condition. Then the
 15 samples were washed by ~~the~~ Hank's liquor ~~for~~ 2 times and nonserum
 16 RPMI1640 liquor ~~for~~ 1 time. Then the clear samples were adjusted to the
 17 2×10^6 /ml cell suspension with 5% FCS-RPMI liquor. 1 ml of the
 18 suspension was added to the round-bottom centrifuge tube. After adding
 19 the ConA (10ng/ml), the samples were cultured in ~~the~~ 5% CO₂ at 37°C
 20 for 72 ~~hour~~hours.

21 The MH60 cells, which grew depending on the IL-6 and were on
 22 the logarithmic growth stage, were adjusted to the 1×10^5 /ml cell
 23 suspension with the 5% FC-RPMI1640.

24 The 96-hole flat bottom cell culturing ~~batten~~wasplate had added
 25 ~~with~~ the MH60 cell suspension ~~on~~at the quantity of 100μl/hole and the
 26 culturing supernate 25μl/ hole. Then the fluid in each hole was adjusted
 27 to ~~the~~ 200μl with the 5% FCS-RPMI 1640. Each sample was cultured

with 3 copies and compared with the different solutions standard rHIL-6 and the pure culturing fluid. The ~~battenplate~~ well plate was cultured in 5%CO₂ at 37°C for 72 hours. 6 hours before the end of the culture, the samples were centrifuged. ~~Each~~ In each hole ~~was sucked out~~, the supernate 110μl was sucked out and ~~added~~ the MTT 10μl was added. The samples were kept at 37°C for 3 hours. And then ~~they were measured~~ the OD at the wavelength 570nm and 630nm were measured. The final OD value = OD 570nm – OD 630nm.

$$\text{IL-6 activity} = \frac{\text{Sample OD} - \text{Culturing Fluid Control OD}}{\text{Standard Sample OD} - \text{Culturing Fluid Control OD}} \times \text{Sample Dilution} \times \text{Activity Of The Standard (IU/ml)}$$

Table 5.2 The effect of Fengshiping on the IL-1, IL-6 ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Mouse number | IL-1 (ng/ml) | IL-6 (IU/ml) |
|---|-------------|--------------|--------------|--------------|
| Control | - | 10 | 78.7±7.1 | 94.6±6.8 |
| | 7.5 | 10 | 59.3±4.9** | 64.9±4.8** |
| | 15 | 10 | 53.3±5.7** | 60.5±4.3** |
| Fengshiping | 30 | 10 | 54.4±4.8** | 56.0±4.6** |
| | 60 | 10 | 47.0±16.6** | 56.6±6.1** |
| <i>Tripterygium hypoglaucum</i> (Levl.) | 5 | 10 | 57.6±4.7** | 65.7±4.9** |
| Hutch. cyclophosphane | 0.02 | 9 | 44.5±7.7 | 49.6±6.7** |

Based on the data in the table 5.2, the Fengshiping had an ~~abvious~~ obvious inhibiting effect on the macrophage in producing of IL-1 and spleen cell in producing IL-6. Along with the increase of the dosage, the effect is ~~enhanced to~~ enhanced.

5.3 The effect of Fenghsiping on the plasma NO in the AA rat

60 SD rats, 160 ~ 220g weight, half male and half female, were divided into 6 groups. The rats in the blank control group were injected the NS 0.5ml under the skin of the right postpede vola. Other rats were

injected with the FCA 0.5ml at the same place as that of the control group. 18 days later, the AA model was built. Then the rats were ~~drenched~~ given orally the ~~correspondent~~ corresponding medicines or the distilled water once a day for 5 days. 3 groups were ~~drenched~~ given orally the solution of Fengshiping ~~on the~~ at a high, middle and low dilution. The positive group was ~~drenched~~ with given orally Glucosidorum Tripterygll Totorum. The blank control group and the model group were ~~drenched~~ with given orally the distilled water of the same volumn. 1 hour after the last administration, each rat ~~was sampled~~ the's blood from the abdominal aorta ~~for~~ was sampled at 2 ml. The plasma of the blood samples ~~were~~ was separated and saved at - 70°C for the measurement. The measurement of NO was done ~~on~~ as per the ~~direction~~ directions of the NO reagent. 0.1ml plasma was added in 0.6ml reagent C and 0.4ml ~~double~~ re-distilled water. After the mixture was shaken up, it was added in 0.1ml reagent D and cultured on the ice for 60 min. Then it was centrifuged at 12000 rpm for 2 min. The supernate was separated. 0.6 ml supernate was mixed with 0.4ml ~~double~~ re-distilled water and 0.1ml reagent A, and then it was cultured in the ice-water for 15 min. Then the mixture was added in reagent B 0.1ml and put at the room tempperature for 1 hour. Then the new mixture OD was measured ~~the OD~~ at the wavelength of 545nm. Based on the OD value of the sample, the content of NO was calculated on the standard curve. (See the result in table 5.3)

Table 5.3 The effect of Fengshiping on the plasma NO level in the AA rat ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Rat number | Content of NO ($\mu\text{mol/L}$) | y ($y=Lgx$) |
|-------|----------------|---------------|--|------------------|
|-------|----------------|---------------|--|------------------|

| | | | | |
|-------------------------|----------------------|---|----------------------------|-------------|
| Control | - | 8 | 13.55±1.11* | 1.131±0.032 |
| AA model | - | 9 | 17.56±4.15 | 1.235±0.097 |
| Fengshiping | 12 | 7 | 9.83±2.58*** [△] | 0.985±0.087 |
| Fengshiping | 24 | 7 | 10.12±1.56*** [△] | 1.001±0.067 |
| Fengshiping | 48 | 7 | 10.70±1.51*** [△] | 1.026±0.062 |
| Glucosidorum Totorum | Tripterygll 0.006 | 7 | 15.25±3.48 | 1.173±0.099 |

Comparing to the model group*P<0.05 , **P<0.01 ; comparing to the Glucosidorum Tripterygll Totorum[△]△P<0.01

Based on the data in table 5.3, the NO level was higher in the model group than in the blank control group. The Fengshiping had an obvious effect on lowering the NO level in the AA rat. The Glucosidorum Tripterygll Totorum had the similar effect but its effect was weaker than that of the Fengshiping.

Experimental example 6 : The effect of Fengshiping on the T lymphocyte, CD₄, CD₈ and NK cells in the mouse.

6.1 The effect of Fengshiping on the ~~transform~~transformation of ~~lymphocyte~~lymphocytes in the normal mouse.

80 NIH mice, half male and half female, were divided into 8 groups randomly and ~~drenched~~with given orally the ~~correspondent~~corresponding medicines once a day for 10 days. 24 hours after the last administration, all the mice were killed to sample the spleen cells aseptically. Then the samples were washed ~~by the~~with Hank's liquor ~~for~~ 2 times and nonserum RPMI1640 liquor ~~for~~ 1 time. Then the clear samples were adjusted to the 2×10⁶/ml cell suspension with 5% FCS-RPMI liquor. The 96-hole flat bottom cell culturing ~~batten~~plate was ~~added~~filled with the cell suspension ~~on~~at the quantity of 100μl/hole. Each sample was cultured with 3 copies. 2 holes were added ~~in~~with 2ng

ConA each as the stimulating reagent. The other hole ~~was~~did not ~~added~~
~~in~~have any additions of the ConA and was kept as the control hole. The
~~batten~~platter was cultured in 5% CO₂ at 37°C for 72 hours. 14 hours
before the end of the culture, each hole ~~was added in~~had 3H-TdR 0.1μCi
added. The cells were harvested by the multihead cell harvesting
instrument and ~~measured~~had the cpm value measured. The average value
was adopted as the sample's cpm value. The average value and the
stimulating index of the different groups were compared directly. The
stimulating index was calculated as following:

$$\text{Stimulating Index} = \frac{\text{Stimulated cpm}}{\text{Control cpm}}$$

See the result in tale 6.1

**Table 6.1 The effect of Fengshiping on the lymphacytolymphecy to
transformation induced by ConA in the mouse ($\bar{X} \pm S$)**

| Group | Dose (g/kg) | Mouse number | cpm | Stimulating index |
|---|--------------------|-----------------|--------------|----------------------|
| Control | - | 10 | 20433±3579 | 25.87±3.06 |
| | 7.5 | 10 | 13566±1779** | 27.29±7.67 |
| Fengshiping | 15 | 10 | 12708±1692** | 18.04±3.76 |
| | 30 | 10 | 12809±2575** | 16.17±4.37 |
| | 60 | 10 | 12090±1706** | 19.05±3.80 |
| <i>Tripterygium hypoglaucum</i> (Levl.) | 2.5 | 10 | 18038±3359 | 17.11±2.60 |
| Hutch. | 5 | 10 | 12081±1039** | 17.58±4.37 |
| Cyclophosphane | 0.02 | 9 | 9922±1145** | 13.66±2.28 |

Comparing to the control group*P<0.05 , **P<0.01

According to the data in table 6.1, it indicated that the Fengshiping
had an obvious inhibiting effect on the lymphocyte transformation and
there was a dosage-effect relationship.

6.2 The effect of Fengshiping on the CD₄, CD₈ and NK cells

The experiment was same ~~to as~~ 5.1. 24 hours after the last administration, the spleen cell samples were made into ~~the a~~ 2×10^8 /ml cell suspension with 5% FCS-RPIM1640. The quantity of CD₄, CD₈, NK cells and the rate CD₄/CD₈ were measured ~~on~~ by the usural method.

The measurement of CD₄ and CD₈:

~~The~~ 50 μ l of the spleen cell suspension ~~50 μ l~~ was added on the glass to made the cell smear. The glass had been coated by the polylysine. The T cell of the mouse was set as the positive control sample. The cell smear was enveloped by the serum of the normal mouse after it was fixed by ~~the~~ acetone. Then the enveloped sample was added with the antibody of CD₄ and CD₈ which were marked by the hominine biotin. It was incubated at 37°C for 2 hours. Then the sample was added ~~with to~~ the avidin labeled by the enzyme and ~~put held~~ still for 10 min. After ~~added with the substrate~~ was added for 10 min, the mixed sample was washed and dyed with the hematoxylin for 2 min. Then the sample was ~~dyhydrated~~ dehydrated with the grade-alcohol and enveloped with ~~gelatin-glycero~~ glycerol. 200 cells in the smear were chosen as the research target under the high power microscope.

$$\text{Content Of Cell} = \frac{\text{Dyed cell number}}{200} \times 100\%$$

The measurement of the NK cell:

The preparation of the EC cell: The spleen cells were sampled in the asepsis condition. Then the samples were washed by ~~the~~ Hank's liquor ~~for~~ 2 times and nonserum RPMI1640 liquor ~~for~~ 1 time. Then the

clear samples were adjusted to the 2×10^8 /ml cell suspension with 5% FCS-RPMI liquor. This cell suspension was used as the EC.

The preparation of the TC cell: The Yack-1 cells, which were sensitive to the mouse NK cell and on the logarithmic growth phase, were adjusted to the 4×10^4 /ml cell suspension. It was the TC.

Measurement: EC and TC, 100 μ l each were added in the 96-hole flat bottom cell culturing ~~battenplate~~. Each sample was cultured with 3 copies and set 2 control samples: EC and TC. (EC control: EC100 μ l + 5% FCS RPMI 1640 100 μ l ; TC control : TC100 μ l + 5% FCS RPMI 1640 100 μ l). The samples were cultured in 5% CO₂ at 37°C for 24 hours. 6 hours before the end of the culturing, the samples were centrifuged and ~~sucked out~~ 110 μ l supernate were sucked out of each hole. And then 10 μ l of the holes ~~MTT~~ were added into the ~~MTT~~ 10 μ l holes. After ~~put~~ setting at 37°C for 3 hours , the mixed samples OD values were measured ~~the OD value at the wavelength~~ wavelengths of 570nm and 630 nm. The OD of each hole=OD570nm - OD630nm.

$$\text{Activity Of NK} = \left(1 - \frac{\text{Sample } \overline{\text{OD}} - \text{EC Control } \overline{\text{OD}}}{\text{TC Control } \overline{\text{OD}}} \right) \times 100 \%$$

Table 6.2 The effect of Fengshipping on the CD4, CD8, NK cell ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Mouse number | CD4 (%) | CD8 (%) | CD4/CD8 | NK |
|-------------------------------|----------------|--------------|--------------|--------------|-------------|----------------------------|
| Control | - | 10 | 20.80±2.94 | 14.80±2.49 | 1.42±0.18 | 40.13±4.89 |
| Fengshipping | 12 | 10 | 19.14±2.91 | 13.43±2.51 | 1.43±0.08 | 31.94±4.52*** [△] |
| | 24 | 10 | 17.30±2.51** | 12.00±2.40 | 1.46±0.16 | 35.36±3.40*** [△] |
| | 36 | 10 | 16.30±2.50** | 11.23±2.94** | 1.49±0.20 | 31.06±3.53*** [△] |
| <i>Tripterygium</i> Hutch. | 8 | 10 | 16.25±2.25** | 11.50±2.45 | 1.44±0.18 | 32.20±2.00** |
| Cyclophosphane | 0.02 | 10 | 11.50±2.50** | 4.10±1.20** | 2.91±0.53** | 23.10±3.66** |

Comparing to the control group*P<0.05 , **P<0.01 ; comparing to the cyclophosphane[△]P<0.01

According to the table 6.2, ~~it is~~ Fengshiping has some inhibiting effect on CD_4 and CD_8 , ~~there~~ was a relation between the ~~dosage~~ dosage and the effect, but the dosage-effect curve was smooth. The effective dosage of Fengshiping on the inhibiting of CD_4 was 24g/kg. The minimum effective dosage on inhibiting the CD_8 was 36g/kg. ~~As the rate of CD_4/CD_8 , the~~ Fengshiping had no obvious effect. on the rate of CD_4/CD_8 . Cyclophosphane had an obvious effect on the inhibiting of the both kind of cells, and the inhibiting effect on the CD_8 was very powerful, which could increase the rate of CD_4/CD_8 magnificently.

As for NK cell, the Fengshiping had a remarkable inhibiting effect, but the dosage-effect relationship was not certain. ~~As~~ At the same ~~while~~ time, the cyclophosphane had shown an obvious inhibiting effect on the NK cell. On the dosage of 20mg/kg, the inhibitiong effect of cyclophosphane was significantly different from that of the Fengshiping ~~on~~ at the 3 dosages: 12, 24 and 36g / kg.

6.3 The effect on the transformation and function of the T lymphacyto in the AA mouse.

NIH mice, 20 ± 2 g weight, were injected with 0.05 ml FCA under the skin of the right postpede vola to build the AA model. The mice in the control group were injected 0.05ml NS at the same place. 3 weeks later, after the AA model was built, all the mice were ~~drenched~~ given orally the ~~correspondent~~ corresponding medicines once a day for 5 days. 5 days later, all the mice were sampled, and the blood was used to make ~~the~~ a blood smear. The smears were dyed by the esterase. Then the smears were observed under ~~the~~ an oil immersion lens to calculate the percent of the positive-dyed cells (it represented the content of the T

cells in the blood). The spleen cells of the mice were sampled ~~the spleen~~
~~cells in the condition of~~ while under anaesthesia and then the cell
samples were prepared ~~to~~ in a single cell suspension. The cell
suspension was washed by PBS and then its supernate ~~were~~ was
abandoned. The rest ~~part was added with~~ had blood cytolysate 4ml
added. The mixed sample was shaken for 2 ~ 3 min to solute the RBC.
After the RBCs were destroyed, the sample was centrifuged to separate
and abandon the supernate. The sample without supernate was washed
by the luminescence lotion for 2 times. Then it was centrifuged to
separate and abandon the supernate. In the next step, the sample was
adjusted to the 1×10^6 /ml cell suspension. Each tube was added with 50 μ l
diluted antibody of CD₄ and CD₈. Then the ~~tub~~ subtubes were cultured at
4°C for 1 hour. After the culture, the samples were washed with the
luminescence ~~lotion for~~ solution 2 times and ~~added in~~ 2ml of the fixing
fluid ~~2 ml was added~~. After fixing, the samples were ~~filtrated~~ filtered
through the 400-mesh screen to the FCA tube. The ~~filtrated~~ filtered
samples were analyzed by the flow cytometer (FCM). The result ~~was~~ is
shown in the table 6.3.

Table 6.3 The effect of Fengshiping on the T cell in the AA mouse

| $(\bar{X} \pm S)$ | | | | | |
|-------------------|----------------|--------------|---------------|---------------|--------------|
| Group | Dose (g/kg) | ANAE+ (%) | CD4+ (%) | CD8 (%) | CD4+/CD8+ |
| Control | - | 50.60±4.25 | 26.13±1.16 | 15.56±0.68 | 1.68±0.03 |
| AA model | - | 49.00±4.22* | 32.56±2.87** | 13.59±1.03** | 2.49±0.16** |
| | 7.5 | 49.13±4.03* | 27.30±1.76##* | 15.98±1.11##* | 1.71±0.04##* |
| Fengshiping | 15 | 49.31±3.29* | 27.96±1.67##* | 16.23±1.27##* | 1.73±0.05##* |
| | 30 | 48.56±3.23* | 26.75±1.94##* | 15.58±1.29##* | 1.72±0.04##* |
| Glucosidorum | 0.012 | 48.88±2.89* | 27.88±1.99##* | 16.33±1.31##* | 1.70±0.03##* |

Tripterygll
Totorum

n=8 , comparing with the control group*P<0.05 , **P<0.01 ;
comparing with the model group# P<0.05 , ## P<0.01 ; comparing with
the control group▲P>0.05

According to the data in table 6.3, there was no significant
difference in the different groups on the ANAE positive cell. But in the
AA mouse, the increase of the CD₄ was significant, while the decrease of
CD₈ was significant too. So the rate of CD₄/CD₈ had a remarkable
increase. The result indicated that the Fengshiping could adjust the CD₄,
CD₈ and CD₄/CD₈ to the normal range.

Experimental example 7: The effect of Fengshiping on the
phagocytic function of the macrophage in the mouse abdominal cavity.

50 NIH mice, 18~ 22g weight, half male and half female, were
divided into 5 groups and ~~drenched with~~given orally the
~~correspondent~~corresponding medicine , solutions ~~on~~at the same
~~volume~~volume. The administration was once a day for 7 days. 1 hour
after the last administration, all the mice were injected with 0.2ml 10 %
chick RBC into the abdominal cavity. 4 hours later, all the mice were
killed and ~~sampled~~the fluid in the abdomincal cavitycavity was
sampled. The liquor samples were dropped on the glass and ~~counted~~the
number of ~~the macrophage~~macrophages were counted which had
phagocytized the CRBC and the number of the CRBC in one
macrophage were also counted. (See the result in table 7)

**Table 7 The effect of Fengshiping on the CRBC phagocytosis
function**

of the macrophage in ICR mouse abdominal cavity ($\bar{X} \pm S$)

| group | dose (g/kg) | Mouse number | Percent of phagocytosis (%) | phagocytosis index |
|---------------|--------------------|-----------------|--------------------------------|-----------------------|
| Control | - | 10 | 25.75±9.40 | 1.28±0.20 |
| Fengshiping | 27 | 10 | 33.20±12.77 | 1.46±0.36 |
| Fengshiping | 40.5 | 10 | 35.20±10.16 | 1.21±0.20 |
| Fengshiping | 60.9 | 10 | 37.78±20.14 | 1.53±0.32 |
| dexamethasone | 0.005 | 10 | 8.33±10.13* | 1.10±0.18 |

*P<0.05

According to the table 7, the Fengshiping had no obvious effect on the phagocytosis function of the macrophage in the mouse abdominal cavity.

Experimental example 8: The effect of Fengshiping on the hyperfunction of the capillary permeability in the mouse abdominal cavity.

90 NIH mice, 18~22g weight, half male and half female, were divided into 9 groups and ~~drenched~~ with given orally the ~~correspondent~~ corresponding medicine solutions of the same ~~volume~~ volume. The medicines were ~~drenched~~ given once a day for 3 days or just 1 time. 1 hour after the last administration, each mouse ~~were~~ was injected with 0.7% HAC – NS solution into the abdominal cavity. At the same time, each mouse was injected with the 0.5% Evans blue – NS solution into the vessel ~~on~~ at the dose of 0.1ml/10 g. 30 min later; all the mice were killed by cervical disjoint. The abdominal cavity was opened and washed ~~by~~ with the 5ml NS. The NS used was collected and adjusted to 8ml by the pure NS as the sample. The samples were centrifuged at 3000 rpm to get the supernate. The supernate OD was measured ~~the OD~~ at the wavelength ~~at~~ of 590nm. (See the result in table 8)

Table 8 The effect of Fengshiping on the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity ($\bar{X} \pm S$)

| Group | Dose (g/kg) | Administration | Mouse number | Leakage of the tincture (OD) | P value |
|---------------|-------------|----------------|--------------|------------------------------|---------|
| Control | - | - | 10 | 0.29±0.13 | |
| Fengshiping | 27 | qd×1 | 10 | 0.26±0.14 | >0.05 |
| Fengshiping | 40 | qd×1 | 10 | 0.25±0.10 | >0.05 |
| Fengshiping | 60 | qd×1 | 10 | 0.25±0.09 | >0.05 |
| Control | - | - | 10 | 0.28±0.15 | |
| Fengshiping | 27 | qd×3 | 10 | 0.25±0.12 | >0.05 |
| Fengshiping | 40 | qd×3 | 10 | 0.18±0.10 | <0.05 |
| Fengshiping | 60 | qd×3 | 10 | 0.15±0.13 | <0.05 |
| dexamethasone | 0.15 | qd×3 | 10 | 0.11±0.07 | <0.01 |

According to the data in table 8, it indicated that Fengshiping could obviously inhibit the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity if it was ~~drenched~~given the medicine for 3 days continuously. If the medicine was ~~drenched~~given for just 1 time, the inhibiting effect was not obvious.

Experimental example 9: The effect of Fengshiping on the pleuritis exudation and the inflammatory cell aggregation induced by the carrageenan.

The mice were divided into 5 groups at random and injected with 0.5% Evans blue NS solution into the caudal vein ~~on~~at the dosage of 0.1ml/10g. Then the mice were injected with the 0.03ml 1% carrageenan in the right chest cavity with the special syringe ~~needle~~needle. 4 hours and 32 hours after the injection, the ~~correspondent~~corresponding mice were killed and ~~open the abdominal~~had their abdominal cavity opened to expose the diaphragm. 2ml of the ~~lotions~~solution were injected to the chest cavity ~~by 2 times~~ with a 1 ml injector. The ~~lotions~~solution was

collected and saved in a test tube. 20 μ l of the ~~lotionsolution~~ lotionsolution collected was added into the 400 μ l WBC dilution. The WBC in the mixed dilution was counted under the microscope. The rest of the ~~lotionsolution~~ lotionsolution was centrifuged at 3000rpm for 10 min. The supernate of the ~~lotionsolution's~~ lotionsolution's OD was measured ~~the OD~~ at the wavelength of 600nm. The OD value of the sample should be corrected with the correspondent OD value of the pure ~~lotionsolution~~ lotionsolution. (See the result in table 9)

Table 9 The effect of Fengshiping on the inflammatory cell aggregation induced by the carrageenan ($\bar{X} \pm S$)

| Group | Dose (g/kg) | WBC number(2×10^5) | | Tincture exudation (OD) | |
|---------------|----------------|-------------------------------|----------------|-------------------------|-------------------|
| | | 4h | 32h | 4h | 32h |
| Control | - | 46.0 \pm 6.9 | 16.0 \pm 9.6 | 0.156 \pm 0.066 | 0.109 \pm 0.019 |
| Fengshiping | 27 | 26.8 \pm 4.5* | 14.2 \pm 8.0 | 0.121 \pm 0.062 | 0.116 \pm 0.031 |
| Fengshiping | 40.5 | 10.9 \pm 4.0** | 17.3 \pm 4.6 | 0.100 \pm 0.048 | 0.153 \pm 0.032 |
| Fengshiping | 60 | 8.0 \pm 5.5** | 6.6 \pm 4.7* | 0.129 \pm 0.066 | 0.092 \pm 0.051 |
| dexamethasone | 0.05 | 12.7 \pm 10.2** | 4.4 \pm 4.0* | 0.085 \pm 0.045 | 0.063 \pm 0.017 |

*P<0.05 , **P<0.01

According to the table 9, it indicated that the Fengshiping had an obvious inhibiting effect on the inflammatory cell aggregation. The effect was powerful at the early stage. The regression equation on the data of the fourth hour was as following follows: $y=44.13 - 2.01x$, $r= - 0.9625$. The effect on the late stage was weak. At the high dosage of 20g/kg, the medicine could affect the aggregation of

the WBC. But it had no obvious effect on the pleuritis exudation.

Experimental example 10: Effect on aggregation of leucocyte in rats' CMC sac.

Sixty four SD rats, 150-180g weight, half male and half female, were randomly divided into 8 groups, which were ~~drenched with~~ given orally the same volume and different dosage of drug liquid once a day, lasting 3 days. A day before experiment, rats were injected with 20ml 1% CMC solution into the sac at the rat's back caused by 20ml air injection before the experiment. 3.5 ~~hour~~ hours and 7.5 ~~hour~~ hours later, 0.1ml of liquid in the sac was extracted each time, and was colored in 0.01% brilliant cresyl blue solution. ~~leucocyte was~~ and leucocytes were counted in the sac liquor under a microscope. The results ~~showed~~ are shown in the table 10.

Table 10 effect on leucocyte counts of carboxymethyl cellulose sac of rats with Fengshiping ($\bar{X} \pm S$)

| groups | dosage (g/kg) | rats number | WBC count($\times 10^7/L$) | |
|-------------|------------------|----------------|------------------------------|-------------------|
| | | | 3.5 hrs | 7.5 hrs |
| control | - | 8 | 9.7 \pm 4.2 | 57.7 \pm 17.3 |
| Fengshiping | 27 \times 1 | 8 | 8.5 \pm 3.5 | 39.4 \pm 16.5 |
| Fengshiping | 40 \times 1 | 8 | 8.7 \pm 7.3 | 35.3 \pm 23.2 |
| Fengshiping | 60 \times 1 | 8 | 6.6 \pm 3.3 | 18.1 \pm 8.6** |
| Control | - | 8 | 10.97 \pm 6.7 | 35.6 \pm 11.2 |
| Fengshiping | 27 \times 3 | 8 | 15.4 \pm 9.7 | 38.6 \pm 15.5 |
| Fengshiping | 40 \times 3 | 8 | 4.8 \pm 3.4** | 18.4 \pm 12.2** |
| Fengshiping | 60 \times 3 | 8 | 3.0 \pm 2.8** | 11.0 \pm 9.2* |
| cortisone | 0.1 \times 3 | 8 | 14.2 \pm 8.0 | 41.7 \pm 16.0 |
| Control | - | 8 | 10.9 \pm 3.0 | 41.3 \pm 6.9 |
| Fengshiping | 18 \times 7 | 8 | 6.2 \pm 3.0* | 11.4 \pm 6.4* |
| Fengshiping | 27 \times 7 | 8 | 3.7 \pm 1.7** | 6.4 \pm 3.1** |
| Fengshiping | 40 \times 7 | 8 | 2.5 \pm 1.9** | 5.9 \pm 3.9** |
| cortisone | 2mg \times 1 | 8 | 1.5 \pm 0.7** | 3.0 \pm 1.0** |

Compared with control group**P<0.01

According to the table 10, the Fengshiping could inhibit significantly, aggregation of leucocyte in the rats' CMC sac, and the inhibition showed apparent dosage-effect ~~relation~~correlation, which was stronger as administration time lasted. With administration of continuing seven days, wandering of leucocyte could be inhibited significantly at dosage of 18g/kg, at the same time, there was also very strong inhibition with cortisone injection into the sac.

Experimental example 11: The effect on croton oil-induced swelling in the ears of mice.

60 NIH mice with weight of 18 ~ 22g, male and female accounting for half and half, were divided into 6 groups, which were ~~drenched~~given orally with the same volume and different dosage of drug liquid or tragacanth liquid, once a day, lasting 3 days. 1 hour after the final administration, 2% croton oil mixture of 0.02ml was embrocated uniformly on ~~the~~ both sides of the left ears of the mice, and after 4 hours, the mice were ~~snapped off its~~put to death by snapping their cervical vertebra ~~and put to death~~. The left and right ears were cut down, then inflammatory and control ears were ~~weighted~~weighed by certain means. ~~Difference~~Differences of weight between left and right ears was the swelling extent of the ears, with results ~~showing~~shown in table 11.

Table 11 ~~effect~~Effect on croton oil-induced swelling of the ears of mice with Fengshiping ($\bar{X} \pm S$)

| Groups | dosage (g/kg) | rats number | Degree of ears' swelling (mg) | inhibition rate (%) | P value |
|---------------|------------------|-------------|-------------------------------|------------------------|---------|
| Control group | - | 10 | 44.38±9.40 | | |
| Fengshiping | 27 | 10 | 39.05±12.33 | 12.00 | >0.05 |
| Fengshiping | 40 | 10 | 36.65±5.83 | 17.64 | <0.05 |

| | | | | | |
|---------------|-------|----|------------|-------|-------|
| Fengshiping | 60 | 10 | 34.91±9.71 | 21.34 | <0.05 |
| dexamethasone | 0.003 | 10 | 14.13±5.75 | 68.16 | <0.01 |

It was ~~As~~ seen from table 11, that Fengshiping had remarkable inhibition to croton oil-induced swelling of the ears of mice, and had ~~quantitydosage-effect relation~~correlation, but ~~whichthe~~ curve was ~~gentleeven~~ and smooth. There was significant inhibition effect at 13.5g/kg of dosage.

Experimental example 12: Effect on acetic acid-induced twisting reaction of mice.

60 Kuming mice with weight of 18~22g, male and female accounting for half and half, were randomly divided into 6 groups, which were ~~drenched~~given orally with different dosages of drug liquid or ~~Xihuangcitragacanth~~ solution. 1 ~~hour~~hour after administration, 0.7% HAC saline of 0.2ml was injected, sc, and the mice were placed in an aquarium and observed the latent period before the twisting reaction of each mouse and the twisting times in 20 minutes, with the results ~~showingshown~~ in table 12:

Table 12 The effect of Fengshiping on acetic acid-induced twisting reaction of mice ($\bar{X} \pm S$)

| groups | dosage (g/kg) | Rats numbers | Twisting times | Latent time (minute) |
|---|------------------|--------------|----------------|-------------------------|
| Control | - | 10 | 34.6±14.1 | 3.13±0.80 |
| Fengshiping | 27 | 10 | 28.2±5.76 | 3.82±0.85 |
| Fengshiping | 40 | 10 | 31.0±18.4 | 3.86±2.00 |
| Fengshiping | 60 | 10 | 20.7±12.3* | 3.95±1.42 |
| Tripterygium hypoglaucom (Levl.) Hutch. | 20 | 10 | 25.1±11.9 | 3.60±0.93 |
| morphine hydrochloride | 10mg/kg | 10 | 0.0±0.0 | 0.00±0.00 |

It was seen from table 12 that large ~~dosedoses~~ of Fengshiping could delay the latent time before the HAC-induced twisting reaction and

1 significantly reduce the twisting times in 20 minutes, which indicated
2 Fengshiping had the effect of ~~abirritation~~aberration in some degree.

3 Experimental example 13: Effect on hemorheology of AA rats.

4 Each of SD rats, 180 ± 20 g weight, were injected intracutaneously
5 with 0.05ml Freund's complete adjuvant on the right back foot
6 metatarsal, and developed into adjuvant arthritis models. Each of the rats
7 of negtive control group were injected intracutaneously with 0.05ml
8 salin on the right back foot metatarsal. Three weeks after models were
9 built, the rats were divided into model group, large, middle, small dosage
10 ~~group~~groups, negtive control group and positive control group which
11 was administered with Glucosidorum Tripterygli Totorum. The rats were
12 ~~drenched~~given the medicines orally once a day, lasting 5 days, 1
13 ~~hour~~hour after administration for the last time, ~~and~~ 3ml of blood was
14 taken from abdominal aorta of rats and placed into test tube with 1%
15 heparin as decoagulant, in which the whole blood viscosity was
16 measured at shear rate of 230, 115, 46, 23, 11.5, 5.75 S^{-1} with an NXE-1
17 cone and plate viscometer. The plasma viscosity was measured with a
18 WTP-BII adjustable constant pressure capillary viscosimeter. The
19 haematocrit, erythrocyte aggregation index was measured with the
20 centrifugation method of packed cell volume. The rigidity index was
21 calculated from the above-mentioned data. All the results ~~showed~~are
22 shown in table 13.

1

2

3

Table 13 Effect on hemorheology of adjuvant arthritis model rats ($\bar{X} \pm S$)

| Groups | Control group | Model group | Fengshiping (30g/kg) | Fengshiping (15g/kg) | Fengshiping (7.5g/kg) | Glucosidorum Tripterygli Totorum (6mg/kg) |
|----------------------------------|---------------|---------------|-------------------------|-------------------------|--------------------------|--|
| whole blood viscosity (mPa.s) | | | | | | |
| 230S-1 | 4.43±0.09 | 4.92±0.15** | 4.56±0.09## | 4.49±0.11## | 4.54±0.16## | 4.66±0.28# |
| 115S-1 | 5.17±0.25 | 5.81±0.19** | 5.33±0.09## | 5.32±0.10## | 5.16±0.14## | 5.60±0.48# |
| 46S-1 | 6.84±0.11 | 7.20±0.18** | 6.56±0.13## | 6.59±0.09## | 6.67±0.14## | 6.70±0.48# |
| 23S-1 | 8.10±0.15 | 8.23±0.38 | 7.95±0.22 | 7.93±0.12 | 7.97±0.14 | 8.02±0.14 |
| 11.5S-1 | 9.35±0.08 | 9.78±0.10** | 9.40±0.08## | 9.45±0.10## | 9.30±0.133 | 9.31±0.12## |
| 6.5S-1 | 11.03±0.14 | 12.66±0.31** | 11.21±0.21## | 11.29±0.19## | 11.60±0.40## | 11.42±0.52## |
| Plasma (mPa.s) | 1.158±0.032 | 1.248±0.040** | 1.161±0.011## | 1.154±0.023## | 1.156±0.018## | 1.158±0.029## |
| corpuscular volume (%) | 46.13±2.31 | 41.33±1.12** | 45.10±2.39## | 44.33±1.52## | 45.71±1.04## | 46.03±3.59## |
| erythrocyte aggregation index | 2.49±0.032 | 2.58±0.083* | 2.46±0.066# | 2.49±0.094# | 2.44±0.048## | 2.45±0.091# |
| rigidity index | 6.155±0.536 | 7.127±0.557** | 6.506±0.558 | 6.525±0.146 | 6.394±0.200# | 6.621±0.883 |

2 Compared with negative control group*P<0.05, **P<0.01; compared with model control group# P<0.05, ##

3 P<0.01

According to the table 13, the hemorheology of AA rats ~~were~~was changed significantly. The whole blood and plasma viscosity increased, haematocrit decreased, aggregation index and ~~rigidity~~rigidity index of erythrocyte increased. The Fengshiping could ~~make~~significantly improve the above-mentioned indexes of hemorheology ~~improved significantly~~.

Pharmacological effects of Fengshiping have been proved by the above-mentioned experiments. Many important pharmacological effects of Fengshiping had favorable dosage-effect ~~relation~~correlation, which implied the best therapeutic effectiveness might be obtained by adjusting the drug dosage at ~~clinical work~~clinic.

The clinical studies on Fengshiping were carried on in China, Japan and Austrilia. Theses studies were operated according to international criterion related disease classification about diagnosis, therapy and curative effect. ~~By using the Fengshiping capsules Sololy, its~~The effective rate for RA was around 94%, and its remarkable effective rate was around 60%. It could improve the symptoms such as morning stiffness, swelling and pain and so on and the related items. The results showed in table 14 ~ 21.

Table 14 Compared effect of treatment group with control group

| Groups | Cases | remission (clinic al recovery) | Notable effect | Effective | No effect | Notable effect rate (%) | Effective rate (%) |
|--------------------|-------|-------------------------------------|-------------------|-----------|--------------|---------------------------------|----------------------------|
| Treatment group | 32 | 5 | 14 | 11 | 2 | 59.38 | 93.74 |
| Control group | 30 | 3 | 10 | 12 | 5 | 43.33 | 83.33 |

Table 15 Influence of IgG, IgA and IgM ($\bar{X} \pm S$)

| Groups | cases | IgG | | IgA | | IgM | |
|-----------------|-------|------------|--------------|-----------|-------------|-----------|-----------|
| | | pre - | post - | pre - | post - | pre - | post - |
| Normal | 32 | 12.45±1.48 | | 2.37±1.00 | | 1.58±0.59 | |
| Treatment group | 32 | 16.92±3.49 | 14.17±1.39** | 3.65±1.03 | 2.39±1.18** | 1.89±0.88 | 1.48±1.01 |
| Control | 30 | 17.03±4.12 | 15.14±2.21** | 3.45±1.86 | 2.32±1.75** | 2.03±0.95 | 1.76±1.28 |

Comparing with pre-treatment **P<0.01

Table 16 Influence of C3 and C4($\bar{X} \pm S$)

| groups | cases | C3 | | C4 | |
|-----------------|-------|-----------|-------------|-----------|-------------|
| | | pre - | post - | pre - | post - |
| normal group | 32 | 0.62±0.13 | | 0.14±0.15 | |
| Treatment group | 32 | 1.88±0.72 | 1.25±0.66** | 0.48±0.12 | 0.26±0.06* |
| Control group | 30 | 2.13±0.64 | 1.56±0.62** | 0.40±0.16 | 0.25±0.07** |

Comparing with before therapy *P<0.05, **P<0.01

Table 17 Influence of ESR and CRP ($\bar{X} \pm S$)

| Groups | cases | ESR | | CRP | |
|-----------|-------|------------|---------------|------------|-------------|
| | | pre- | post- | pre- | post- |
| Normal | 32 | 8.37±5.26 | | 4.12±1.88 | |
| Treatment | 32 | 66.58±9.01 | 30.31±6.53** | 13.35±6.67 | 8.86±3.34* |
| control | 30 | 73.33±9.09 | 35.83±11.61** | 14.21±6.29 | 9.04±3.15** |

Comparing with pre-treatment *P<0.05, **P<0.01

Table 18 Compared with power of gripping pre- and post-treatment

($\bar{X} \pm S$)

| groups | Treatment Group | | Control Group | |
|---------------------------------------|-----------------|-------------------|-----------------|-------------------|
| | pre - | post - | pre - | post - |
| Gripping power of left hands (mmHg) | 39.13±20.24(15) | 80.47±34.61**(15) | 24.00±17.63(21) | 55.15±23.27**(21) |
| Right hands | 35.85±22.46(15) | 85.32±36.32**(15) | 22.80±12.32(21) | 58.17±20.59**(21) |

Comparing with pre-treatment *P<0.05, **P<0.01

Table 19 Influence of arthrosis swelling and pain and morning stiffness time ($\bar{X} \pm S$)

| Items | Treatment Group | | Control Group | |
|-----------------------------------|-----------------|--------------|---------------|--------------|
| | pre - | post - | pre - | post - |
| arthrosis swelling and pain | 5.79±0.52 | 3.14±0.83* | 5.56±2.15 | 3.92±0.26* |
| morning stiffness time (minute) | 50.33±6.47 | 20.24±3.27** | 48.75±8.34 | 27.50±3.78** |

Comparing with pre-treatment *P<0.05, **P<0.01

Table 20 Influence of RF changing to negative

| Groups | Cases | RF negative | | |
|-----------------|-------|-----------------|------------------|---------------------------------|
| | | Pre - treatment | Post - treatment | Rate of negative turnaround (%) |
| Treatment group | 32 | 24 | 11 | 54.2 |
| Control group | 30 | 18 | 10 | 44.4 |

Not only ~~haddid~~ it have significant effects, but also Fengshiping can make items such as SIL-2R, STNF, SIL-6R in plasma decrease, results showing in the Table 21.

Table 21 inflence of main indes such as SIL - 2R, STNF and SIL - 6R ($\bar{X} \pm S$)

| groups | Cases | SIL - 2R(u/ml) | | STNF R1(ng/ml) | | SIL - 6R(ng/ml) | |
|-------------|-------|------------------|-----------|---------------------|--------------------|-----------------------|---------------|
| | | pre - | post - | pre - | post - | pre - | post - |
| Normal | 32 | 299±68 (n=32) | | 1.56±0.48 (n=24) | | 72.05±18.26 (n=22) | |
| Fengshiping | 15 | 683±189 | 381±157** | 2.87±0.66 | 1.75±0.54** | 136.18±28.57 | 90.15±20.12** |
| Control | 10 | 765±203 | 412±167** | 2.63±0.72 | 2.38±0.39 (n=8) | 148.21±30.31 | 99.02±26.70** |

Comparing with pre-treatment **P<0.01

It was proved that the above-mentioned results ~~on~~ of the invention could be realized ~~on~~ by the ways as ~~following~~ follows.

Practice example 1:

Example of exploitation 1:

Epimedium brevicornum Maxim. 2222g

Tripterygium hypoglaucum (Levl.) Hutch. 2222g

Lycium barbarum L. 1111g

Cuscuta chinensis Lam. 1111g

~~Four herbs hereinbefore,~~ *Tripterygium hypoglaucum* (Levl.) Hutch. was cut into pieces, extracted for three times ~~after~~with 13, 10, 10-fold ~~added in~~times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim was cut into segments, extracted three times ~~after~~with 15, 10, 10-fold~~times~~ water was added in, each ~~extraction~~time lasting 1 hour; *Lycium barbarum* L. was crushed ~~to raw material~~into coarse powder, and immersed in 20-fold~~times~~ water of 80°C for 1 hour; *Cuscuta chinensis* Lam. was crushed ~~to raw~~into coarse powder, immersed in 31-fold~~times~~ water of 80°C for 1 hour; the decoction fluid or immersion fluid of four herbs were filtrated ~~repectively~~respectively, poured across macropore polymeric adsorbent ~~column~~resin columns, and eluted with 70% ~~ethanol~~alcohol. When the color of effluent became deep significantly, eluent ~~was commenced~~started to ~~collect~~be collected; when the color of effluent became very weak, elution collection was ended. ~~Eluent~~The alcohol in the eluent of each herb was ~~reecyelled to get ethanol~~recovered. Then the fluid without alcohol was concentrated, dried to get the finally ~~extractive drug~~extract powder; officinal starch was blended with the four kinds of ~~drug~~extract powder to 200g, mixed up uniformly and encapsuled into 1000 capsules. Each capsule which was prepared with the invented method thereof, was composed of 0.2g ~~drugs~~extractive drug extract and contained at least 2.0mg of ~~icariine~~icariin C₃₃H₄₀O₁₅. The

regular dosage is: oral administration, three times every day, three capsules ~~every~~for each time.

Practice example 2:

Example of exploitation 2:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

~~Two herbs hereinbefore,~~ *Tripterygium hypoglaucum* (Levl.) Hutch. ~~were~~was cut into pieces, extracted three times ~~after~~with 13, 10, 10-fold ~~added in~~times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim. was cut into segments, extracted three times ~~after~~with 15, 10, 10-fold ~~water was added in, each extraction~~times water, each time lasting 1 hour; decoction fluid of herbs were filtrated ~~repectively~~respectively, poured across macropore polymeric adsorbent resin column, eluted with 70% ethanol, when the color of effluent became deep significantly, eluent was commenced to collect; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was reecyled to get ethanol, concentrated, dried, finally extractive drug powder was obtained~~The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariin C₃₃H₄₀O₁₅. regular dosage is: oral administration, three times every day, three capsules for each time.

Example of exploitation 3:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; *Lycium barbarum* L. was crushed to coarse powder, and immersed in 20-times water of 80°C for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated respectively, poured across a macropore polymeric adsorbent column, eluted with 70% alcohol, when the color of effluent became deep significantly, eluent was started to be collected; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariine $C_{33}H_{40}O_{15}$. regular dosage is: oral administration, three times every day, three capsules every time.

Practice example 3:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

~~*Lycium barbarum* L. 1000g~~*Tripterygium hypoglaucum* (Levl.)

~~Hutch. were cut into pieces, extracted three times after 13, 10, 10 fold added in, each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times after 15, 10, 10 fold water was added in, each extraction lasting 1 hour; *Lycium barbarum* L. was~~

crushed to raw material, and immersed in 20 fold water of 80°C for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated repectively, poured across macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, eluent was commenced to collect; when the color of effluent became very weak, elution was over. Eluent of each herbs was reeyeled to get ethanol, concentrated, dried, finally extractive drug powder was obtained; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariine $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules ~~every~~for each time.

Practice example 4

Example of exploitation 4

Tripterygium hypoglaucum (Levl.) Hutch. 2000g

Epimedium brevicornum Maxim. 2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~werewas~~was cut into pieces, extracted three times ~~after~~with 13, 10, 10-fold ~~added in~~times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim. was cut into segments, extracted three times ~~after~~with 15, 10, 10-fold ~~times~~times water ~~was added in~~, each ~~extraction~~time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to raw ~~coarse~~coarse powder, immersed in 31-fold ~~times~~times water of 80°C for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated ~~repectively~~respectively, poured across a macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent

became deep significantly, collection of eluent was commenced to collect ~~begin~~; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol,~~ The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried, to get the finally extractive drug extract powder was obtained; officinal starch was blended with ~~extractive~~ extract drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g ~~drugs~~ extractive drug extract, contains at least 2.0mg of ~~icariine~~ icariin $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules ~~every~~ for each time.

Practice example 5

Example of exploitation 5

Tripterygium hypoglaucum (Levl.) Hutch. 2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. ~~Were~~ was cut into pieces, extracted three times ~~after~~ with 13, 10, 10-fold ~~added in~~ times water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to ~~raw~~ coarse powder, immersed in 31-fold ~~times~~ times water of 80°C for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated ~~repectively~~ respectively, poured across the macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent was commenced to collect ~~begin~~; when the color of effluent became very weak, elution was over. ~~Eluent of each herbs was recycled to get ethanol,~~ The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried, to get the finally extractive drug extract powder was

1 ~~obtained~~; officinal starch was blended with extractive drug powder, and
2 mixed up uniformly, and loaded to 1000 capsules. ~~Dose~~The dose of
3 capsules administered every day, which was prepared with the inventive
4 method thereof, was equivalent to ~~dose of~~ 30g/day of crude drugs.

5 Practice example 6:

6 Example of exploitation 6:

7 *Tripterygium hypoglaucum* (Levl.) Hutch. 2000g

8 *Lycium barbarum* L. 1000g

9 *Tripterygium hypoglaucum* (Levl.) Hutch. ~~were~~was cut into pieces,
10 extracted three times ~~after~~with 13, 10, 10-fold ~~added~~intimes water, each
11 time lasting 1 hour; *Lycium barbarum* L. was crushed to ~~raw~~
12 ~~material~~coarse powder, and immersed in 20-fold~~times~~ water of 80°C for
13 1 hour; decoction fluid or immersion fluid of the herbs were filtrated
14 ~~repectively~~respectively, poured across macropore polymeric adsorbent
15 column, eluted with 70% ethanol, when the color of effluent became
16 deep significantly, collection of the eluent ~~was commenced to~~
17 ~~collect~~began; when the color of effluent became very weak, elution was
18 over. ~~Eluent of each herbs was reecycled to get ethanol,~~The alcohol in the
19 eluent of each herb was recovered. Then the fluid without alcohol was
20 concentrated, dried, to get the finally ~~extractive drug powder was~~
21 ~~obtained~~extract powder; officinal starch was blended with extractive
22 drug powder, and mixed up uniformly, and loaded to 1000 capsules.
23 ~~Dose~~The dose of capsules administered every day, which was prepared
24 with the inventive method thereof, was equivalent to ~~dose of~~ 30g/day of
25 crude drugs.

Claims

~~1. A pharmaceutical composition for treating rheumatism, characterized in that, it is made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch.~~

~~Epimedium brevicornum Maxim.~~

~~Lycium barbarum L.~~

~~Cuscuta chinensis Lam., Cuscuta australis R. Br.~~

~~Wherein the materials must be composed of Tripterygium hypoglaucum (Levl.) Hutch and one or two or three other herbs in the rest 3 herbs.~~

~~2. The pharmaceutical composition according to claim 1 made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch. 1-4 part by weight~~

Epimedium brevicornum Maxim. 1-4 part by weight

Lycium barbarum L. 1-4 part by weight

~~Cuscuta chinensis Lam., Cuscuta australis R. Br. 1-4 part by weight.~~

~~3. The pharmaceutical composition according to claim 1 made from the following materials:~~

~~Tripterygium hypoglaucum (Levl.) Hutch. 2 part by weight~~

~~Epimedium brevicornum Maxim. 2 part by weight~~

~~Lycium barbarum L. 1 part by weight~~

~~Cuscuta chinensis Lam., Cuscuta australis R. Br. 1 part by weight~~

~~4. The pharmaceutical composition according to claim 1, characterized in that, it can be made from the correspond effective constituents of the materials above mentioned as following that Epimedium brevicornum Maxim. can be replaced by any one or more than one among icariine, deuteron icariine I, deuteron icariine II and glyc icariine A; Tripterygium hypoglaucum (Levl.) Hutch can be replaced by diterpenoids, triterpenoids and alkaloids compound thereof, and Lycium barbarum L. and Cuscuta chinensis Lam. can be replaced by flavone contained thereof.~~

~~5. A method of preparing the pharmaceutical composition according to claim 1, 2 or 3, characterized in that, it includes the processes under mentioned:~~

~~The raw herbs are weighed, and Epimedium brevicornum Maxim. and Tripterygium hypoglaucum (Levl.) Hutch. were cut into pieces respectively; including raw material or crushed powder of Lycium barbarum L. and Cuscuta chinensis Lam., four herbs hereinbefore, were extracted with 0-95% ethanol at 10-98°C respectively or combinatively for continuing 1-4 times. Ethanol was recycled respectively or~~

1 ~~combinatively in extracted fluid, then extraction was concentrated, dried,~~
2 ~~crushed, mixed uniformly or proportionally, manufactured to dosage~~
3 ~~form adopted in clinical work;~~

4 ~~—Raw herbs were weighed: Epimedium brevicornum Maxim. and~~
5 ~~Tripterygium hypoglaucum (Levl.) Hutch. were cut into pieces, boiled~~
6 ~~out in water for three times respectively, and Lycium barbarum L. or~~
7 ~~Cuscuta chinensis Lam. were immersed in water of 80°C ~ 95°C for 1-3~~
8 ~~times respectively. Decoction or immersion fluids of three times of each~~
9 ~~herb were blended respectively, then mixture fluid was respectively~~
10 ~~poured through corresponding macropore polymeric adsorbent column.~~
11 ~~After absorption, resin column was washed with water until effluent~~
12 ~~became clear, then was eluted with 30-99.5% ethanol until color of~~
13 ~~effluent became deep. Then eluent was collected until color of eluent~~
14 ~~became from deep to very weak while ethanol liquid was forced out~~
15 ~~from the column with water. Eluent was mixed with the ethanol liquid.~~
16 ~~The weight of total eluent was 1-8 fold of the herbs; eluent of each herbs~~
17 ~~was recycled, concentrated to specific gravity of 1.10 respectively, then~~
18 ~~extractive of every herbs were obtained by respective or combinative~~
19 ~~spray drying, which were mixed uniformly and proportionally,~~
20 ~~manufactured to dosage form adopted in clinical work.~~

21 ~~6. A method of preparing the pharmaceutical composition according to~~
22 ~~claim 1, 2 or 3, characterized in that, it can be made into any dose forms~~
23 ~~adopted in the clinical work such as hard gelatin capsule, soft capsule,~~
24 ~~tablet, granule and injection.~~

25 ~~—7. A method of preparing the pharmaceutical composition according to~~
26 ~~claim 1, 2 or 3, characterized in that, it includes the processes under-~~
27 ~~mentioned:~~

1 ~~Tripterygium hypoglaucum (Levl.) Hutch. were cut into pieces,~~
2 ~~extracted three times after 13, 10, 10 fold added in respectively, each~~
3 ~~time lasting 1 hour; Epimedium brevicornum Maxim. was cut into~~
4 ~~segments, extracted three times after 15, 10, 10 fold water was added in~~
5 ~~respectively, each extraction lasting 1 hour; Lycium barbarum L. was~~
6 ~~crushed to raw material, and immersed in 20 fold water of 80°C-95°C~~
7 ~~for 1 hour; Cuscuta chinensis Lam. was crushed to raw powder,~~
8 ~~immersed in 31 fold water of 90°C for 1 hour; decoction fluid or~~
9 ~~immersion fluid of four herbs were filtrated repectively, poured through~~
10 ~~WLD or D₁₀₁ or other type of macropore polymeric adsorbent column,~~
11 ~~eluted with 70% ethanol, when the color of effluent became deep~~
12 ~~significantly, eluent was commenced to collect; when the color of~~
13 ~~effluent became very weak, elution was over. Eluent of each herbs was~~
14 ~~recycled to get ethanol, concentrated, dried, finally extractive drug~~
15 ~~powder was obtained; which were mixed uniformly and proportionally,~~
16 ~~manufactured to dosage form adopted in clinical work.~~

17 ~~8. The use of the pharmaceutical composition according to claim 1, 2 or~~
18 ~~3 in the manufacture of a medicament for treating the rheumatoid and~~
19 ~~rheumatoid arthritis.~~

20 ~~—9. The use of the pharmaceutical composition according to claim 1, 2 or 3~~
21 ~~in the manufacture of a medicament for treating the systemic lupus~~
22 ~~erythematosus.~~

23 ~~—10. The use of the pharmaceutical composition according to claim 1, 2 or 3~~
24 ~~in the manufacture of a medicament for treating the chronic nephritis,~~
25 ~~crohn's disease and lepra reaction and the other autoimmune disease.~~

Abstract

~~The invention has brought to light a kind of~~An antirheumatic medicine and its preparation, ~~which was~~are disclosed. The medicine is made from *Tripterygium hypoglaucum* (Levl.) Hutch, *Epimedium brevicornum* Maxim, *Lycium barbarum* L, and *Cuscuta chinensis* Lam. The ~~invented~~ medicine has the merits of prominent ~~effect~~therapeutic efficacy, mild side ~~reaction~~reactions, and convenient administration.

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